Official Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

NCT Number: NCT00924703

Applicant/MAH: BioMarin Pharmaceutical Inc.

Version Date: 31 March 2017

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical Affairs BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Until product is commercially available or until study is terminated
Dose:	0.001 to 1.0 mg/kg per individual injection, with a maximum weekly dose of 2.0 mg/kg
Date of Original Protocol:	October 08, 2008

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 8 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve central nervous system developmental and functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (Study PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU who participated in Study PAL-002.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

STUDY DESIGN AND PLAN:

This is a long-term extension of a Phase 2, open-label, dose-finding study (Study PAL-002) in

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

approximately 35 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety.

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

A Data Monitoring Committee will monitor the study.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 participants' rAvPAL-PEG dosing will continue in PAL-003 without interruption of weekly dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations below 480 µmol/L. Subjects will continue to receive injections in the clinic, administered by clinic staff, but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug doses will be evaluated on an individual basis.

The dose may be modified at any time for safety.

Evaluations, observations, and procedures will be conducted at selected timepoints as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been controlled to within a target range (below 480 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The drug becomes commercially available following the appropriate marketing approval.
4. The study is terminated.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Dose Adjustment Methodology:

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Depending upon the response to rAvPAL-PEG in PAL-002, a subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration.
- When adjusting the dose, a dose may be used that is between the dose cohort levels that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency of injections per week, up to one dose per day, for a maximum total dose per week of 2 mg/kg.
- When a dose is adjusted (either by changing the dose level or by changing the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.

Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made, so that blood Phe concentrations can be assessed.

Stopping Criteria:

If an individual subject exhibits drug-related toxicity of CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit drug-related toxicity of CTCAE grade 3 or greater, further dosing of subjects at that dose level and any higher dose levels will be halted until a safety assessment is completed.

NUMBER OF SUBJECTS PLANNED:

Approximately 35 subjects.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

- Must have completed participation in PAL-002.
- Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.
- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

- Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Known hypersensitivity to rAvPAL-PEG or its excipients.
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

rAvPAL-PEG doses will be administered SC and the favorable dose (the dosage that provides control of blood Phe concentrations within the target range for a minimum of 2 weeks) will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.

DURATION OF TREATMENT:

Extension of multiple dosing will continue until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The drug becomes commercially available following the appropriate marketing approval.
4. The study is terminated.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:
None

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs or laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured when steady-state levels of Phe are attained.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

Safety Analysis:

All subjects who receive any amount of study drug in this study will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any posttreatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled timepoint will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled timepoint will also be summarized.

Pharmacokinetic Analysis:

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
the same day each week for 3 consecutive weeks.		

2.1 Schedule of Events

Table 2.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]				Final F/U or ETV
		Week 1	Weekly	Monthly (eg, Wk 4, 8, etc,	Qrtly (eg, Wk 12, 24, etc)	4 weeks after final dose
		D 1				
Informed consent	X					
Medical history and demographics	X					
Physical examination[d]	X	X			X	X
Vital signs	X	X	X	X	X	X
12-lead ECG	X					X
Clinical laboratory tests[e]	X			X	X	X
Sedimentation rate		X			X	X
Urine pregnancy test	X	X		X	X	X
Injection-site inspectionf		X (postdose)	X	X	X	X
Adverse events[g]	X←----------→ X					
Concomitant medications	X	X	X	X	X	X
Diet query	X	X	X	X	X	X
Serum rAvPAL-PEG antibodies		X		X	X	X
Predose blood Phe and plasma tyrosine[h]	X	X	X	X	X	X
Blood Phe (fingerstick)[i]			3 days postdose			
Predose PK sample[j]						
Administer IP[k]		X	X	X	X	

AE, adverse event; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] Screening assessments must be completed within 28 days from Day 1. These may be the same used for the Final Follow-up Visit of PAL-002, if they occur ≤ 28 days from Day 1.

[b] Additional visits may occur if deemed necessary to monitor AEs or blood Phe, adjust dosing etc.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Complete physical examinations to include the evaluation of all major body systems, height (at Screening only), and weight.

[e] Clinical laboratory tests to include hematology, chemistry, and urinalysis.

f If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, e.g. right arm vs. left arm)

[g] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[h] Samples should be drawn in the morning, at least 2.5 hours after a meal. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

[i] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

[j]. The PK blood samples will be collected after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks. Once this target is achieved, predose PK samples will be collected on the same day each week for 3 consecutive weeks as specified in Section 9.7.3.

[k] Dosing is up to 1.0 mg/kg per injection and up to one dose per day, for a total of up to 2.0 mg/kg/week.

3 TABLE OF CONTENTS

LIST OF TABLES

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_{trough}	predose concentration of rAvPAL-PEG
CD	Compact disk
CFR	Code of Federal Regulations
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CTCAE	Common Terminal Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DCF	Data Clarification Form
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit

FDA	Food and Drug Administration
f/u	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
IgG	immunoglobulin G
IgM	immunoglobulin M
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IND	Investigational New Drug
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase

PEG	methoxypolyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	phenylalanine ammonia lyase
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR §50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The murine model of phenylketonuria (PKU) BTBR*Pah*^{enu2} (ENU^2), a mouse line that is deficient in phenylalanine hydroxylase (PAH) activity (Shedlovsky, 1993, Genetics), was used for in vivo screening and pharmacodynamic studies. This mouse model exhibits characteristics similar to those seen in PKU patients, including hyperphenylalaninemia (baseline plasma phenylalanine [Phe] concentrations of 1000 to 2000 µM) and hypopigmentation. Weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG (approximately 4 IU/mouse) for greater than 3 months lowered blood Phe concentration from approximately 2000 µM to less than 200 µM. A similar profile of Phe reduction was also seen in ENU^2 mice administered wild-type AvPAL-PEG (4 IU/mouse) over 8 weeks. In all these studies, regardless of the molecule, an attenuated Phe-lowering response was usually seen between Weeks 2 and 7. However, plasma Phe concentrations became stabilized at concentrations below 200 µM from Week 7 onward. In addition to the reduction of plasma Phe, a dose-related darkening in coat color occurred, indicating the biosynthesis of melanin, which was previously inhibited when plasma Phe could not be metabolized and plasma Phe concentrations were high. When administration of AvPAL-PEG was stopped for 2 to 7 weeks and then reinitiated with weekly administrations, no attenuated response was observed and Phe concentrations remained low throughout the week.

The safety of rAvPAL-PEG was evaluated in safety pharmacology studies (respiratory, central nervous system [CNS] and cardiovascular [CV]) and toxicity with toxicokinetic studies (single and 28-day repeated dose) in rats and cynomolgus monkeys. Overall, in all toxicity studies no immune-related toxicities were seen either systemically or at the injection site. No specific methoxypolyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies.

Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included morbidity, decreased plasma Phe concentration, decreased protein synthesis, and gastrointestinal (GI) lesions. Plasma Phe concentrations were also reduced to below the level of detection in the lower dose groups, but without similar

toxicological consequence. Physiologically regulated Phe intake may have played a part in these morbidity results, although plasma Phe concentrations were low in all treated monkeys.

The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups.

The half-life of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg, was 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose linearity. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the half-life was 65 hours at 4 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum plasma concentration, along with a long half-life, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) AUC and the maximum plasma concentration (C_{max}) are roughly linearly proportional.

7.2 Previous Clinical Studies

This study is an extension of the second human clinical study with rAvPAL-PEG (Study PAL-002). Study PAL-001, the first-in-human clinical study of rAvPAL–PEG, was designed as a Phase 1, open-label, single-dose, dose-escalation study in approximately 35 subjects, 16 to 50 years old, with PKU. The doses for this study are planned to be in the same range as those tested in PAL-001 and PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in those studies.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH (Scriver CR, 2001, McGraw-Hill). Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver CR, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver CR, 2001, McGraw-Hill). The brains of untreated patients

with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve central nervous system developmental and functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from PAL-001 and PAL-002 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to PEG

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given up to 60 mg/kg of SC rAvPAL-PEG.

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to Immunologic Sensitization

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. In animal models, the immune response against the foreign protein PAL in rAvPAL-PEG has been mitigated by PEGylation. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers, sedimentation rates, and complete blood counts (CBCs).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.) (Harris, 2003, Nat.Rev.Drug Discov.), and antibodies against PEG seem to form only under special circumstances (Harris, 2003, Nat.Rev.Drug.Discov.) (Richter, 1983, Int.Arch.Allergy Appl.Immunol.) and may be of no clinical relevance in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.).

Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate, or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 2 hours after injection. Longer observations may be required at the discretion of the PI. The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection.

7.4.2.1 Management of Allergic Reactions

The following measures are recommended for the treatment of allergic symptoms:

- Clearing the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. Acetaminophen or ibuprofen (5-10 mg/kg) may also be administered. An allergy and/or immunology consultation should be sought if necessary.

7.4.3 Effects of Low Blood Phe

Insufficient Phe intake or excessive rAvPAL-PEG exposure may cause blood Phe concentrations to be too low (eg, < 30 μmol/L). Prolonged low blood Phe concentrations can result in a catabolic state associated with poor growth and altered body functions, including mental and physical alterations, loss of appetite, anemia, rashes, and diarrhea. To ensure safety during this study, subjects will be monitored closely with frequent blood Phe concentration determinations.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU who participated in Study PAL-002.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of a Phase 2, open-label, dose-finding study (PAL-002) in approximately 35 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety.

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

A Data Monitoring Committee will monitor the study.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 participants' rAvPAL-PEG dosing will continue in PAL-003 without interruption of weekly dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations below 480 µmol/L based upon recommendations by the German Working Group for Metabolic Diseases (Burgard, 1999, Eur J Pediatr). Subjects will continue to receive injections in the clinic, administered by clinic staff, but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug doses will be evaluated on an individual basis. All of the following criteria must be met before a subject may self-administer doses of the study drug:

- The subject is willing and able to administer the study drug as directed.
- The sponsor agrees that self-administration is appropriate.
- The subject has achieved a favorable dose (ie, the dosage that provides control of blood Phe concentrations within the target range for a minimum of 2 weeks).
- The subject has received appropriate training, including administering doses under supervision, until the subject and clinic staff both agree such training is no longer necessary.

The dose may be modified at any time for safety.

Evaluations, observations, and procedures will be conducted at selected timepoints as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been

controlled to within a target range (below 480 μmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The drug becomes commercially available following the appropriate marketing approval.
4. The study is terminated.

9.1.1 Dose Adjustment Methodology

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Depending upon the response to rAvPAL-PEG in PAL-002, a subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration.
- When adjusting the dose, a dose may be used that is between the dose cohort levels that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency of injections per week, up to one dose per day, for a maximum total dose of 2 mg/kg/week.
- When a dose is adjusted (either by changing the dose level or by changing the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.

Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made so that blood Phe concentrations can be assessed.

9.1.2 Stopping Criteria

If an individual subject exhibits drug-related toxicity of CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision

will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit drug-related toxicity of CTCAE grade 3 or greater, further dosing of subjects at that dose level and any higher dose levels will be halted until a safety assessment is completed.

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

- Must have completed participation in PAL-002.
- Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.
- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
- Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Known hypersensitivity to rAvPAL-PEG or its excipients.
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject experiences a serious or intolerable AE.
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Each subject was assigned a unique subject identifier in either PAL-001 or PAL-002. Subjects will retain the same subject number used in PAL-002.

This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

9.4 Treatments

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Dilution instructions are provided in a separate instruction manual.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

At the study site, all IP must be stored at 5 ±3°C (41 ± 5°F) under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 2 mg/kg.

Dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous

measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

The injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in PAL-002.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU.
A favorable dose that produces a reduction in blood Phe concentration to below 480 µmol/L will be determined for each subject. Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included severe morbidity, decreased plasma Phe concentration, decreased protein synthesis, and GI lesions. Plasma Phe concentrations were reduced to below the level of detection in the lower dose groups, without other findings. The doses for this study will be based upon the results of PAL-001 and PAL-002.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered in the morning by clinic staff or by the subject, if appropriate.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any

contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in PAL-002) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study except as necessary for safety.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis (Section 2.1) describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.5
rAvPAL-PEG-specific antibodies	BioMarin	9.7.4.2
PK variables	Central laboratory	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1

Phe, phenylalanine; PK, pharmacokinetic.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety. This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of weight; general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Height will be measured at Screening only. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the final follow-up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn in the morning, at least 2.5 hours after a meal, on the days and timepoints indicated in the Schedule of Events (Table 2.1.1).

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.

BioMarin will perform the analysis.

9.7.4 Safety Variables

9.7.4.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the timepoints specified in the Schedules of Events (Table 2.1.1). Female subjects with a positive pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test results.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.4.2 rAvPAL-PEG-specific Antibodies

Immunoglobulin G (IgG) and M (IgM) antibodies and neutralizing antibodies (NAb) to rAvPAL-PEG will be measured using validated immunogenicity assays at the timepoints indicated in the Schedules of Events (Table 2.1.1).

BioMarin will perform the analysis.

9.7.5 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Specific days for obtaining samples are provided in Table 2.1.1 and in Section 12. The scheduled clinical laboratory tests are listed in Table 9.7.5.1. A central laboratory will be used for analysis.

Table 9.7.5.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Pharmacokinetics
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Serum rAvPAL-PEG-specific antibodies (IgG, IgM, and NAb)
BUN	Differential cell count	Protein	
Creatinine	Sedimentation rate	Glucose	
GGT		Bilirubin	
Total protein		Nitrite	
Calcium		Urobilinogen	
Sodium		Hemoglobin	
Potassium			
Glucose			
Uric acid			
CO_2			
Chloride			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; IgG, immunoglobulin G; IgM, immunoglobulin M; NAb, neutralizing antibodies; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

According to the ICH definition, an AE (or adverse experience) is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the IP.”

An adverse drug reaction (ADR) is described by the ICH as “all noxious and unintended responses to a medicinal product related to any dose.” This means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, ie, the relationship cannot be ruled out.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The reporting period for non-serious AEs is the period from the first administration of study drug through the final F/U Visit or at the ETV. If a non-serious AE remains unresolved at the conclusion of the study, the PI and medical monitor will make a joint clinical assessment as to whether continued follow-up of the AE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

The Investigator will assess AEs for severity, for relationship to IP, and as to whether the event meets one or more of the definitions of an SAE (refer to Section 10.2).

The Investigator will determine the severity of each AE and will record it on the source documents and AE CRF, using the categories defined below.

Grade	Description
Mild	No limitation of usual activities
Moderate	Some limitation of usual activities
Severe	Inability to carry out usual activities

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the relationship of an AE to the IP and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	**Description**
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A SAE is defined as any AE that:

- Results in death.
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization.
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, that is, an AE that occurs in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Is an important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

More than one of the above criteria may apply to any specific event.

The reporting period for SAEs begins earlier than non-serious AEs and is the period from the time of signing of the ICF through 4 weeks after the last dose or at the ETV. SAEs reported to the Investigator outside of this reporting period will be reported to BioMarin if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

Any SAE, whether or not considered related to study drug, will be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate CRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying BioMarin Pharmacovigilance of an SAE. BioMarin may be required to

report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by BioMarin as soon as it becomes available.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history and demographics
- Physical examination and height
- Vital signs
- 12-lead ECG
- Clinical laboratory tests (including hematology, chemistry, and urinalysis)
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration

For subjects who participated in PAL-002, these assessments may be the same used for the Final Follow-up Visit of PAL-002, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done on the third day following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week [up to 1 dose per day] as determined by the Investigator, in agreement with the Study Medical Officer)

12.3.2 Weekly Visits

The following study activities will be performed at the weekly visits beginning with Week 2:

- Vital signs
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasm tyrosine concentration
- Administer study drug (study drug may be administered on additional days during the week [up to 1 dose per day] as determined by the Investigator, in agreement with the Study Medical Officer)

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.3 Monthly Visits (Week 4, 8, 16, etc)

The following study activities will be performed at the monthly visits beginning with Week 4:

- Vital signs
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week [up to 1 dose per day] as determined by the Investigator, in agreement with the Study Medical Officer)

12.3.4 Quarterly Visits (Week 12, 24, 36, etc)

Quarterly visits consist of all monthly activities and include additional activities. The following study activities will be performed at the quarterly visits beginning with Week 12:

- Physical examination
- Vital signs
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration

- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week [up to 1 dose per day] as determined by the Investigator, in agreement with the Study Medical Officer)

12.4 Final Follow-up Visit (Day 113 [± 3 days]) /Early Termination Visit

The final follow-up (F/U) Visit or Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the F/U Visit or ETV:

- Physical examination and weight
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs and clinically significant changes in vital signs and laboratory test results.

The verbatim terms reported on CRFs to identify AEs will be coded using MedDRA. Treatment-emergent AEs will be summarized for each treatment group by system organ class, preferred term, relationship to study drug, and severity. Changes from baseline in vital signs and laboratory test results will be summarized with descriptive statistics by dose group.

14.3 Pharmacokinetic Analysis

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks and no further dose modification is planned.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any posttreatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled timepoint will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled timepoint will also be summarized.

14.5 Determination of Sample Size

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act in an advisory capacity to BioMarin to monitor subject safety and the efficacy of rAvPAL-PEG in subjects who participate in the study. Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Study Medical Officer. The Chair will be notified by the Study Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study and offer advice on whether or not to close the study on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping rules as defined in study protocol.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the

clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of BioMarin, I confirm that BioMarin, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

[redacted] 10/16/2008

Medical Monitor Date

Printed name: [redacted]

BIOMARIN

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Study Title:	Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	**Phase 2**
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical Affairs BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Until product is commercially available or until study is terminated
Dose:	0.001 to 1.0 mg/kg per individual injection, with a maximum weekly dose of 2.0 mg/kg
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	**February 09, 2009**

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 1

Date: February 09, 2009

RATIONALE AND SUMMARY OF CHANGES

The protocol has been amended as follows:

1. Prohibited the use of polyethylene glycol (PEG)-containing injectable medications, because of reported unexpected adverse events (AEs) after intramuscular Depo-Provera® injection in 2 subjects.

- Excluded subjects taking or planning to take PEG-containing injectable medications.
- Added a list of PEG-containing injectable medications as an appendix to the protocol. This list is subject to change.

2. Provided details regarding the 2 subjects who experienced a study drug injection-site reaction and developed unexpected hypersensitivity AEs after a Depo-Provera injection (15 and 40 days, respectively) after the rAvPAL-PEG injection.
3. Added the provision that subjects who experience an allergic reaction may be asked to see an allergist/immunologist and/or provide additional blood samples for antibody testing.
4. Clarified that subjects may require more than 1 injection per dose, depending upon site policy.
    - Added a table as an example.
5. [redacted]
6. Added that at least one interim analysis may be performed by the Sponsor.
7. In interactive voice response system (IVRS) will be track subjects' assigned dose and study drug inventory.
8. Provided clarifying language to allow subjects to complete PAL-002 study and enroll into PAL-003 without study drug interruption.
9. Specified there will be replacement of subjects in this study if any single cohort (identified during PAL-002) drops to less than 3 subjects after the initial enrollment of 35.

10. Made revisions to correct spelling, spacing, or punctuation, and/or made minor editorial revisions to improve clarity and consistency.
11. PAL-001 and/or PAL-002 data may become available during this study and indicate the need for pharmacokinetic measurement of study drug accumulation (in addition to the current plan for analysis of study drug exposure). If this is the case, the PAL-003 protocol will be amended to require additional blood draws for PK analysis.

PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale. Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Comments	Relates to Change No.
Section 2 Synopsis (Study Rationale)	**STUDY RATIONALE**: The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve ~~central nervous system developmental and~~ functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study will build upon the results of the first single-dose pharmacokinetics (PK) study (Study PAL-001) and evaluate whether weekly injections of rAvPAL-PEG can reduce blood Phe concentrations in PKU subjects and whether repeated administration is safe.		10
Section 2 Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: • Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments. • Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.		1

Section No./Title	Revision	Comments	Relates to Change No.
	• Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation. • A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. • Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease). • Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.		
Table 2.1.1 Schedule of Events (footnote a)	[a] It is preferable to perform PAL-003 Screening assessments the same day as PAL-002 Final Follow-up Visit so there is no interruption of study drug. If performed on the same day, PAL-002 Final Follow-up Visit data may be used. If not, PAL-003 Day 1 must occur ≤ 28 days after PAL-003 Screening.		8

Section No./Title	Revision	Comments	Relates to Change No.
Section 4 List of Abbreviations and Definitions of Terms	IM intramuscular	Added new term	10
Section 4 List of Abbreviations and Definitions of Terms	IVRS interactive voice response system	Added new term	7
Section 4 List of Abbreviations and Definitions of Terms	PEG ~~methox~~ypolyethylene glycol		10
Section 7.1 Nonclinical Studies	When administration of AvPAL-PEG was stopped for 2 to 7 weeks and then reinitiated with weekly administrations, no attenuated response was observed and Phe concentrations remained low and stable until the next administration~~throughout the week~~.	Paragraph 1	10
Section 7.1 Nonclinical Studies	No specific ~~methoxy~~polyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies.	Paragraph 2	10
Section 7.1 Nonclinical Studies	Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included ~~morbidity,~~ reduced food consumption, dehydration, body weight loss, hypoactivity, hypothermia,decreased plasma Phe concentration, decreased protein synthesis, and gastrointestinal (GI) lesions. Plasma Phe concentrations were also reduced to below the level of detection in the ~~lower~~ 4 and 12 mg/kg dose groups, but without similar toxicological consequence, implying that the physiological regulation of Phe levels may be an important factor for influencing morbidity. ~~Physiologically regulated Phe intake may have played a~~	Paragraphs 4 and 5	10

Section No./Title	Revision	Comments	Relates to Change No.
	~~part in these morbidity results, although plasma Phe concentrations were low in all treated monkeys.~~ The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups. No degeneration of the arteries was observed in animals administered 0 and 0.01 mg/kg rAvPAL-PEG.		
Section 7.3 Study Rationale	The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood Phe concentrations and improve ~~CNS developmental and~~ functional outcomes. rAvPAL-PEG is a potential substitute for the PAH enzyme. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study will build upon the results of PAL-001 and evaluate whether weekly injections of rAvPAL-PEG can reduce blood Phe concentrations in PKU subjects and whether repeated administration is safe.		10

Section No./Title	Revision	Comments	Relates to Change No.
Section 7.4.2 Toxicity Due to an Immunologic Reaction	PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.) (Harris, 2003, Nat.Rev.Drug Discov.), ~~and~~ however, antibodies against PEG ~~seem to form only under special circumstances~~ may form when PEG is bound to compounds. (Harris, 2003, Nat.Rev.Drug.Discov.) (Richter, 1983, Int.Arch.Allergy Appl.Immunol.) ~~and may be of no~~ In some instances, development of such antibodies did not result in any significant clinical ~~relevance~~ effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.). Two subjects enrolled in PAL-001 who had received one dose of rAvPAL-PEG experienced hypersensitivity reactions to intramuscular (IM) Depo-Provera, a contraceptive containing PEG 3350. Both subjects had previously received multiple IM Depo-Provera injections without complications. The first subject, a [redacted] year old [redacted], developed bruising at the injection site on Day 1 that resolved on Day 15. [redacted] developed hives and mild respiratory difficulty 10 minutes after receiving IM Depo-Provera and 40 days after [redacted] rAvPAL-PEG injection. [redacted] was treated in the emergency room with [redacted]. The events resolved the same day, and the subject had no further complications. The subject had no prior history of any reactions to Depo-Provera. Subsequently, while under the care of an allergist/immunologist, the subject had a mild reaction to a concentrated intradermal (1:10) exposure of Depo-Provera. [redacted] was later given a full dose of IM Depo-Provera without problems.		2, 3, 10

Section No./Title	Revision	Comments	Relates to Change No.
	The second subject, an [redacted] year old [redacted], had a slightly raised, erythematic rash below the site of rAvPAL-PEG injection on Day 11 that resolved without treatment on Day 15. [redacted] developed generalized hives on [redacted] arms and legs and mild difficulty breathing 20 minutes after receiving IM Depo-Provera (and [redacted] third dose of Gardasil) given 15 days after [redacted] rAvPAL-PEG injection. [redacted] symptoms subsided after [redacted] received [redacted] given by [redacted] mother. [redacted] did not visit the emergency room. The subject had no prior history of any reactions to Depo-Provera or Gardasil (Gardasil dose not contain PEG). [redacted] was subsequently seen by an allergist/immunologist, and the results are pending. Because Depo-Provera contains PEG 3350 as an excipient, it is possible that the administration of rAvPAL-PEG caused anti-PEG antibody formation that led to a cross-reaction with Depo-Provera in these subjects. Therefore, it is possible that prior exposure to rAvPAL-PEG might sensitize subjects to other PEG-containing injectable drugs. Alternatively, the presence of Depo-Provera could have led to antibody formation and cross-reaction with rAvPAL-PEG, leading to rAvPAL-PEG injection-site reactions. Since it is currently unknown whether Depo-Provera has a sensitizing effect to PEG and the exact basis for these reported events is not known, the use of PEG-containing injectable drugs prior to and during the study is prohibited as a precautionary measure (refer to the addendum and Sections 9.3.2 and 9.4.8).		
	In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for antibody testing. This additional testing may occur up to 6 months following the final study visit.		

Section No./Title	Revision	Comments	Relates to Change No.
Section 9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG	**9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG** **9.1.1.1 Subjects Who Have Experienced Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product** Subjects who had a previous reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from PAL-001 or PAL-002 are excluded from this study. However, subjects may develop such reactions after enrollment in PAL-003. Management of such reactions are described below. Refer to Figure 9.1.1.1.2.1: for a flowchart for managing these subjects. **9.1.1.2.1 Subjects with Local Reactions** After enrollment in PAL-003, subjects who have a local reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophpen and antihistamines and/or oral steroids, 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (Claritin is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Claritin or 25 to 50 mg oral Benadryl; an example for steroids would be 70 mg oral prednisone). Because Benadryl can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver. **9.1.1.2.2 Subjects with Systemic Reactions** After enrollment in PAL-003, subjects who have a systemic reaction that includes noncutaneous systemic symptoms, such as respiratory problems, hypotension, or angioedema, to rAvPAL-PEG or a PEG-containing product will be evaluated with a skin prick test. If the test is positive, the subject will be excluded from the study. Subjects with prior systemic reactions that included only skin manifestations to rAvPAL-PEG or a PEG-containing product after PAL-003 enrollment will be premedicated with acetaminophen and antihistamines and/or oral steroids (one hour prior to study drug dosing) and will continue to receive rAvPAL-PEG.	Added new section	5

Section No./Title	Revision	Comments	Relates to Change No.
	Reactions to skin prick testing or rAvPAL-PEG administered SC are **defined as follows:** **Local reaction: 1 affected location, ie, hives, wheals, or swelling or an area of erythema or redness at or near the site of injection.** **Systemic reaction: more than 1 affected location, ie, cutaneous reaction in more than 1 area, and/or any other generalized symptoms, such as hypotension, angioedema or anaphylaxis or the involvement of other organ systems (eg, respiratory, cardiovascular, gastrointestinal).**		
Section 9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG	Figure 9.1.1.2.1 Management of Local and System Reactions to rAvPAL-PEG	Added new figure	5
Section 9.3.2 Exclusion Criteria	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: • Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments. • Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.		1

Section No./Title	Revision	Comments	Relates to Change No.
	• A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. • Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug. • Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study. • Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease). • Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.		
Section 9.3.4 Subject Identification and Replacement of Subjects	After enrollment of 35 subjects, if any single cohort (identified during PAL-002) has less than 3 subjects due to early terminations, those subjects will be replaced. Replacement subjects will enter the study at the same dose cohort level as the subject they are replacing.		9

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td>Section 9.4.4
Directions for Administration</td><td>Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.1 as an example. Note this table is for example purposes only. Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual.
Table 9.4.4.1: Number of Injections Required For an Individual Weighing 80 kg
<table>
<tr><th>Dose Group (mg/kg)</th><th>Weight (kg)</th><th>Volume of Study Drug (mL)[a]</th><th>No. of Injections[b]</th></tr>
<tr><td>0.001</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.003</td><td>80</td><td>0.6</td><td>1</td></tr>
<tr><td>0.01</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.03</td><td>80</td><td>0.2</td><td>1</td></tr>
<tr><td>0.1</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.3</td><td>80</td><td>2.4</td><td>2</td></tr>
<tr><td>1.0</td><td>80</td><td>8.0</td><td>4</td></tr>
</table>
[a]Study drug is supplied at a concentration of 10 mg/mL and is then diluted by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume attained following dilution.
[b]Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.</td><td></td><td>4</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
Section 9.4.5 Method of Assigning Subjects to Treatment Groups	An interactive voice response system (IVRS) will be used to track subjects' assigned dose and study drug inventory.		7
Section 9.4.6 Selection of Doses Used in the Study	The doses for this study will be based upon the ~~results of~~ findings in PAL-001 and PAL-002.		10
Section 9.4.8 Prior and Concomitant Medications	Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines and/or oral steroids (refer to Section 9.1.1). Subjects who have had a prior hypersensitivity reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines and/or oral steroids (refer to Section 9.1.1). If the local or systemic reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study.		1
9.7.4.2 rAvPAL-PEG-specific antibodies Antibody Testing	Anti-PEG antibodies will also be measured.	Re-named section	10

Section No./Title	Revision	Comments	Relates to Change No.
Section 14.3 Pharmacokinetic Analysis	Should data become available from PAL-001 and/or PAL-002 which indicate study drug accumulation should also be measured, the protocol will be amended to require additional blood draws for PK analysis.		11
Section 14.7 Interim Analyses	At least one interim analysis may be performed by the sponsor during the study.	Added new section	6

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 8 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (Study PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU who participated in Study PAL-002.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

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STUDY DESIGN AND PLAN:

This is a long-term extension of a Phase 2, open-label, dose-finding study (Study PAL-002) in approximately 35 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety.

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

A Data Monitoring Committee will monitor the study.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 participants' rAvPAL-PEG dosing will continue in PAL-003 without interruption of weekly dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations below 480 µmol/L. Subjects will continue to receive injections in the clinic, administered by clinic staff, but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug doses will be evaluated on an individual basis.

The dose may be modified at any time for safety.

Evaluations, observations, and procedures will be conducted at selected timepoints as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been controlled to within a target range (below 480 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The drug becomes commercially available following the appropriate marketing approval.
4. The study is terminated.

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Dose Adjustment Methodology:

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Depending upon the response to rAvPAL-PEG in PAL-002, a subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration.
- When adjusting the dose, a dose may be used that is between the dose cohort levels that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency of injections per week, up to one dose per day, for a maximum total dose per week of 2 mg/kg.
- When a dose is adjusted (either by changing the dose level or by changing the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.

Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made, so that blood Phe concentrations can be assessed.

Stopping Criteria:

If an individual subject exhibits drug-related toxicity of CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit drug-related toxicity of CTCAE grade 3 or greater, further dosing of subjects at that dose level and any higher dose levels will be halted until a safety assessment is completed.

NUMBER OF SUBJECTS PLANNED:

Approximately 35 subjects.

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DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

- Must have completed participation in PAL-002.
- Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.
- **Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.**

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

- **A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product.**
- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Known hypersensitivity to rAvPAL-PEG or its excipients**, including hypersensitivity reactions that necessitated early termination from PAL-001 or PAL-002.**
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

rAvPAL-PEG doses will be administered SC and the favorable dose (the dosage that provides control of blood Phe concentrations within the target range for a minimum of 2 weeks) will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.

DURATION OF TREATMENT:

Extension of multiple dosing will continue until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The drug becomes commercially available following the appropriate marketing approval.
4. The study is terminated.

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REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:
None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs or laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured when steady-state levels of Phe are attained.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

Safety Analysis:

All subjects who receive any amount of study drug in this study will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any posttreatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled timepoint will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled timepoint will also be summarized.

Pharmacokinetic Analysis:

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.		

2.1 Schedule of Events

Table 2.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]				Final F/U or ETV
		Week 1	Weekly	Monthly (eg, Wk 4, 8, etc,	Qrtly (eg, Wk 12, 24, etc)	4 weeks after final dose
		D 1				
Informed consent	X					
Medical history and demographics	X					
Physical examination[d]	X	X			X	X
Vital signs	X	X	X	X	X	X
12-lead ECG	X					X
Clinical laboratory tests[e]	X			X	X	X
Sedimentation rate		X			X	X
Urine pregnancy test	X	X		X	X	X
Injection-site inspectionf		X (postdose)	X	X	X	X
Adverse events[g]	X←----------→ X					
Concomitant medications	X	X	X	X	X	X
Diet query	X	X	X	X	X	X
Serum rAvPAL-PEG antibodies		X		X	X	X
Predose blood Phe and plasma tyrosine[h]	X	X	X	X	X	X
Blood Phe (fingerstick)[i]			3 days postdose			
Predose PK sample[j]						
Administer IP[k]		X	X	X	X	

AE, adverse event; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] **It is preferable to perform PAL-003 Screening assessments the same day as PAL-002** Final Follow-up Visit **so there is no interruption of study drug. If performed on the same day, PAL-002 Final Follow-up Visit data may be used. If not, PAL-003 Day 1 must occur** ≤ 28 days **after PAL-003 Screening.**

[b] Additional visits may occur if deemed necessary to monitor AEs or blood Phe, adjust dosing etc.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Complete physical examinations to include the evaluation of all major body systems, height (at Screening only), and weight.

[e] Clinical laboratory tests to include hematology, chemistry, and urinalysis.

f If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, e.g. right arm vs. left arm)

[g] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[h] Samples should be drawn in the morning, at least 2.5 hours after a meal. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

[i] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

[j]. The PK blood samples will be collected after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks. Once this target is achieved, predose PK samples will be collected on the same day each week for 3 consecutive weeks as specified in Section 9.7.3.

[k] Dosing is up to 1.0 mg/kg per injection and up to one dose per day, for a total of up to 2.0 mg/kg/week.

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_{trough}	predose concentration of rAvPAL-PEG
CD	Compact disk
CFR	Code of Federal Regulations
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CTCAE	Common Terminal Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DCF	Data Clarification Form
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
f/u	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure

ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	Investigational New Drug
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
IVRS	interactive voice response system
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	phenylalanine ammonia lyase
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified

SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR §50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The murine model of phenylketonuria (PKU) BTBR*Pah*^{enu2} (ENU^2), a mouse line that is deficient in phenylalanine hydroxylase (PAH) activity (Shedlovsky, 1993, Genetics), was used for in vivo screening and pharmacodynamic studies. This mouse model exhibits characteristics similar to those seen in PKU patients, including hyperphenylalaninemia (baseline plasma phenylalanine [Phe] concentrations of 1000 to 2000 µM) and hypopigmentation. Weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG (approximately 4 IU/mouse) for greater than 3 months lowered blood Phe concentration from approximately 2000 µM to less than 200 µM. A similar profile of Phe reduction was also seen in ENU^2 mice administered wild-type AvPAL-PEG (4 IU/mouse) over 8 weeks. In all these studies, regardless of the molecule, an attenuated Phe-lowering response was usually seen between Weeks 2 and 7. However, plasma Phe concentrations became stabilized at concentrations below 200 µM from Week 7 onward. In addition to the reduction of plasma Phe, a dose-related darkening in coat color occurred, indicating the biosynthesis of melanin, which was previously inhibited when plasma Phe could not be metabolized and plasma Phe concentrations were high. When administration of AvPAL-PEG was stopped for 2 to 7 weeks and then reinitiated with weekly administrations, no attenuated response was observed and Phe concentrations remained low **and stable until the next administration**.

The safety of rAvPAL-PEG was evaluated in safety pharmacology studies (respiratory, central nervous system [CNS] and cardiovascular [CV]) and toxicity with toxicokinetic studies (single and 28-day repeated dose) in rats and cynomolgus monkeys. Overall, in all toxicity studies no immune-related toxicities were seen either systemically or at the injection site. No specific polyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies.

Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included **reduced food consumption, dehydration, body weight loss, hypoactivity, hypothermia,**decreased plasma Phe concentration, decreased protein synthesis, and gastrointestinal (GI) lesions. Plasma Phe concentrations were also reduced to below the level of detection in the **4 and 12 mg/kg** dose groups, but without similar

toxicological consequence, **implying that the physiological regulation of Phe levels may be an important factor for influencing morbidity**.

The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups. **No degeneration of the arteries was observed in animals administered 0 and 0.01 mg/kg rAvPAL-PEG.**

The half-life of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg, was 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose linearity. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the half-life was 65 hours at 4 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum plasma concentration, along with a long half-life, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) AUC and the maximum plasma concentration (C_{max}) are roughly linearly proportional.

7.2 Previous Clinical Studies

This study is an extension of the second human clinical study with rAvPAL-PEG (Study PAL-002). Study PAL-001, the first-in-human clinical study of rAvPAL–PEG, was designed as a Phase 1, open-label, single-dose, dose-escalation study in approximately 35 subjects, 16 to 50 years old, with PKU. The doses for this study are planned to be in the same range as those tested in PAL-001 and PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in those studies.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH (Scriver CR, 2001, McGraw-Hill). Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver CR, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and

behavioral abnormalities (Scriver CR, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from PAL-001 and PAL-002 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to PEG

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given up to 60 mg/kg of SC rAvPAL-PEG.

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to an Immunologic Reaction

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. In animal models, the immune response against the foreign protein PAL in rAvPAL-PEG has been mitigated by PEGylation. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers, sedimentation rates, and complete blood counts (CBCs).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.) (Harris, 2003, Nat.Rev.Drug Discov.), **however,** antibodies against PEG **may form when PEG is bound to compounds**. (Harris, 2003, Nat.Rev.Drug.Discov.) (Richter, 1983, Int.Arch.Allergy Appl.Immunol.) **In some instances, development of such antibodies did not result in any significant** clinical **effects** in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.).

Two subjects enrolled in PAL-001 who had received one dose of rAvPAL-PEG experienced hypersensitivity reactions to intramuscular (IM) Depo-Provera, a contraceptive containing PEG 3350. Both subjects had previously received multiple IM Depo-Provera injections without complications.

The first subject, a [redacted] year old [redacted], developed bruising at the injection site on Day 1 that resolved on Day 15. [redacted] developed hives and mild respiratory difficulty 10 minutes after receiving IM Depo-Provera given 40 days after [redacted] rAvPAL-PEG injection. [redacted] was treated in the emergency room with [redacted], and [redacted]; and oral [redacted]. The events resolved the same day, and the subject had no further complications. The subject had no prior history of any reactions to Depo-Provera. Subsequently, while under the care of an allergist/immunologist, the subject had a mild reaction to a concentrated intradermal (1:10) exposure of Depo-Provera. [redacted] was later given a full dose of IM Depo-Provera without problems.

The second subject, an [redacted] year old [redacted], had a slightly raised, erythematic rash below the site of rAvPAL-PEG injection on Day 11 that resolved without treatment on Day 15. [redacted] developed generalized hives on [redacted] arms and legs and mild difficulty breathing 20 minutes after receiving IM Depo-Provera (and [redacted] third dose of Gardasil) given 15 days after [redacted] rAvPAL-PEG injection. [redacted] symptoms subsided after [redacted] received [redacted] given by [redacted] mother. [redacted] did not visit the emergency room. The subject had no prior history of any reactions to Depo-Provera or Gardasil (Gardasil dose not contain PEG). [redacted] was subsequently seen by an allergist/immunologist, and the results are pending.

Because Depo-Provera contains PEG 3350 as an excipient, it is possible that the administration of rAvPAL-PEG caused anti-PEG antibody formation that led to a cross-reaction with Depo-Provera in these subjects. Therefore, it is possible that prior exposure to rAvPAL-PEG might sensitize subjects to other PEG-containing injectable drugs. Alternatively, the presence of Depo-Provera could have led to antibody formation and cross-reaction with rAvPAL-PEG, leading to rAvPAL-PEG injection-site reactions. Since it is currently unknown whether Depo-Provera has a sensitizing effect to PEG and the exact basis for these reported events is not known, the use of PEG-containing injectable drugs prior to and during the study is prohibited as a precautionary measure (refer to the addendum and Sections 9.3.2 and 9.4.8).

Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or

anaphylaxis. Clinical assessments should be conducted for a minimum of 2 hours post-injection. Longer observations may be required at the discretion of the PI. The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection.

In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for antibody testing. This additional testing may occur up to 6 months following the final study visit.

7.4.2.1 Management of Allergic Reactions

The following measures are recommended for the treatment of allergic symptoms:

- Clearing the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. Acetaminophen or ibuprofen (5-10 mg/kg) may also be administered. An allergy and/or immunology consultation should be sought if necessary.

7.4.3 Effects of Low Blood Phe

Insufficient Phe intake or excessive rAvPAL-PEG exposure may cause blood Phe concentrations to be too low (eg, < 30 ˜m ol/L). Prolonged low blood Phe concentrations can result in a catabolic state associated with poor growth and altered body functions, including mental and physical alterations, loss of appetite, anemia, rashes, and diarrhea. To ensure safety during this study, subjects will be monitored closely with frequent blood Phe concentration determinations.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU who participated in Study PAL-002.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of a Phase 2, open-label, dose-finding study (PAL-002) in approximately 35 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety.

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

A Data Monitoring Committee will monitor the study.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 participants' rAvPAL-PEG dosing will continue in PAL-003 without interruption of weekly dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations below 480 µmol/L based upon recommendations by the German Working Group for Metabolic Diseases (Burgard, 1999, Eur J Pediatr). Subjects will continue to receive injections in the clinic, administered by clinic staff, but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug doses will be evaluated on an individual basis. All of the following criteria must be met before a subject may self-administer doses of the study drug:

- The subject is willing and able to administer the study drug as directed.
- The sponsor agrees that self-administration is appropriate.
- The subject has achieved a favorable dose (ie, the dosage that provides control of blood Phe concentrations within the target range for a minimum of 2 weeks).
- The subject has received appropriate training, including administering doses under supervision, until the subject and clinic staff both agree such training is no longer necessary.

The dose may be modified at any time for safety.

Evaluations, observations, and procedures will be conducted at selected timepoints as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been

controlled to within a target range (below 480 μmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses.

A subject will continue in PAL-003 until one of the following occurs:

12. The subject withdraws consent and discontinues from the study.
13. The subject is discontinued from the study at the discretion of the Investigator.
14. The drug becomes commercially available following the appropriate marketing approval.
15. The study is terminated.

9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG

9.1.1.1 Subjects Who Have Experienced Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product

Subjects who had a previous reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from PAL-001 or PAL-002 are excluded from this study. However, subjects may develop such reactions after enrollment in PAL-003. Management of such reactions are described below. Refer to Figure 9.1.1.1.2.1 for a flowchart for managing these subjects.

9.1.1.1.1 Subjects with Local Reactions

After enrollment in PAL-003, subjects who have a local reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophpen and antihistamines and/or oral steroids, 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (Claritin is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Claritin or 25 to 50 mg oral Benadryl; an example for steroids would be 70 mg oral prednisone). Because Benadryl can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver.

9.1.1.1.2 Subjects with Systemic Reactions

After enrollment in PAL-003, subjects who have a systemic reaction that includes noncutaneous systemic symptoms, such as respiratory problems, hypotension, or angioedema, to rAvPAL-PEG or a PEG-containing product will be evaluated with a skin prick test. If the test is positive, the subject will be excluded from the study.

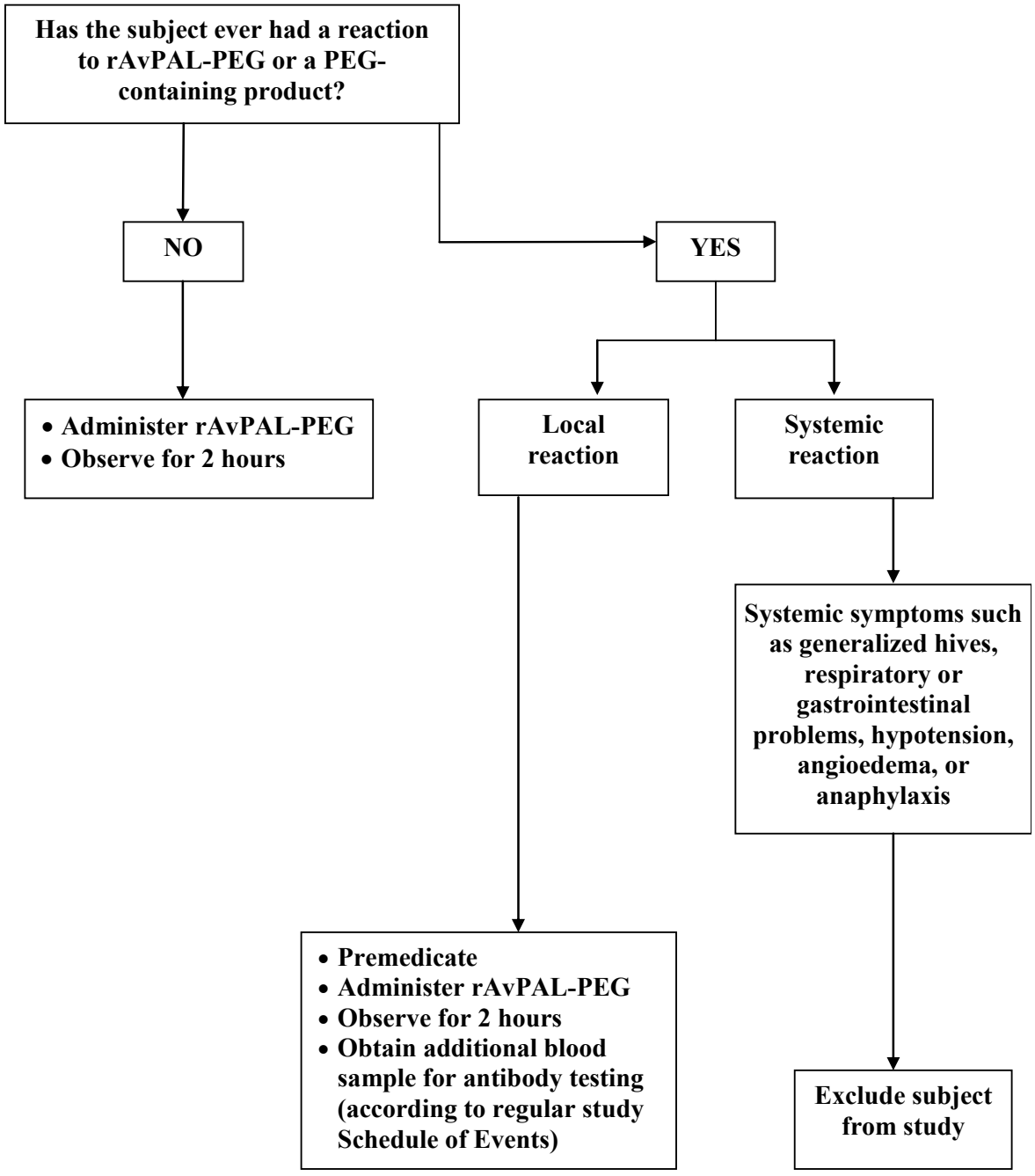
Subjects with prior systemic reactions that included only skin manifestations to rAvPAL-PEG or a PEG-containing product after PAL-003 enrollment will be premedicated with acetaminophen and antihistamines and/or oral steroids (one hour prior to study drug dosing) and will continue to receive rAvPAL-PEG.

Reactions to skin prick testing or rAvPAL-PEG administered SC are defined as follows:

Local reaction: 1 affected location, ie, hives, wheals, or swelling or an area of erythema or redness at or near the site of injection.

Systemic reaction: more than 1 affected location, ie, cutaneous reaction in more than 1 area, and/or any other generalized symptoms, such as hypotension, angioedema or anaphylaxis or the involvement of other organ systems (eg, respiratory, cardiovascular, gastrointestinal).

Figure 9.1.1.1.2.1: Management of Local and Systemic Reactions to rAvPAL-PEG



9.1.2 Dose Adjustment Methodology

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Depending upon the response to rAvPAL-PEG in PAL-002, a subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration.
- When adjusting the dose, a dose may be used that is between the dose cohort levels that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency of injections per week, up to one dose per day, for a maximum total dose of 2 mg/kg/week.
- When a dose is adjusted (either by changing the dose level or by changing the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.

Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made so that blood Phe concentrations can be assessed.

9.1.3 Stopping Criteria

If an individual subject exhibits drug-related toxicity of CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit drug-related toxicity of CTCAE grade 3 or greater, further dosing of subjects at that dose level and any higher dose levels will be halted until a safety assessment is completed.

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

- Must have completed participation in PAL-002.
- Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- **Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.**
- **A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product.**
- Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.

- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
- Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject experiences a serious or intolerable AE.
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Each subject was assigned a unique subject identifier in either PAL-001 or PAL-002. Subjects will retain the same subject number used in PAL-002.

This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

After enrollment of 35 subjects, if any single cohort (identified during PAL-002) has less than 3 subjects due to early terminations, those subjects will be replaced. Replacement subjects will enter the study at the same dose cohort level as the subject they are replacing.

9.4 Treatments

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Dilution instructions are provided in a separate instruction manual.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

At the study site, all IP must be stored at 5 ± 3°C (41 ± 5°F) under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 2 mg/kg.

Dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

The injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper

arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.1 as an example, Note this table is for example purposes only. Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual.

Table 9.4.4.1 Number of Injections Required For an Individual Weighing 80 kg

Dose Group (mg/kg)	Weight (kg)	Volume of Study Drug (mL)[a]	No. of Injections[b]
0.001	80	0.8	1
0.003	80	0.6	1
0.01	80	0.8	1
0.03	80	0.2	1
0.1	80	0.8	1
0.3	80	2.4	2
1.0	80	8.0	4

[a]**Study drug is supplied at a concentration of 10 mg/mL and is then diluted by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume attained following dilution.**

[b]**Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.**

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in PAL-002.

An interactive voice response system (IVRS) will be used to track subjects' assigned dose and study drug inventory.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU.

A favorable dose that produces a reduction in blood Phe concentration to below 480 µmol/L will be determined for each subject. Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included severe morbidity, decreased plasma Phe concentration, decreased protein synthesis, and GI lesions. Plasma Phe concentrations were reduced to below the level of detection in the lower dose groups, without other findings. The doses for this study will be based upon the **findings in** PAL-001 and PAL-002.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered in the morning by clinic staff or by the subject, if appropriate.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in PAL-002) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines and/or oral steroids (refer to Section 9.1.1).

Subjects who have had a prior hypersensitivity reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines and/or oral steroids (refer to Section 9.1.1). If the local or systemic reaction worsens with a repeat injection (as determined by the Investigator in consultation with the

Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study.

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study except as necessary for safety.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis (Section 2.1) describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.5
Antibody testing	BioMarin	9.7.4.2
PK variables	Central laboratory	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1

Phe, phenylalanine; PK, pharmacokinetic.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety. This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of weight; general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Height will be measured at Screening only. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury

(mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the final follow-up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn in the morning, at least 2.5 hours after a meal, on the days and timepoints indicated in the Schedule of Events (Table 2.1.1).

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.

BioMarin will perform the analysis.

9.7.4 Safety Variables

9.7.4.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the timepoints specified in the Schedules of Events (Table 2.1.1). Female subjects with a positive pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test results.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.4.2 Antibody Testing

Immunoglobulin G (IgG) and M (IgM) antibodies and neutralizing antibodies (NAb) to rAvPAL-PEG will be measured using validated immunogenicity assays at the timepoints indicated in the Schedule of Events (Table 2.1.1). **Anti-PEG antibodies will also be measured.**

BioMarin will perform the analysis.

9.7.5 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Specific days for obtaining samples are provided in Table 2.1.1 and in Section 12.
The scheduled clinical laboratory tests are listed in Table 9.7.5.1. A central laboratory will be used for analysis.

Table 9.7.5.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Pharmacokinetics
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Serum rAvPAL-PEG-specific antibodies (IgG, IgM, and NAb)
BUN	Differential cell count	Protein	
Creatinine	Sedimentation rate	Glucose	
GGT		Bilirubin	Phenylalanine
Total protein		Nitrite	
Calcium		Urobilinogen	
Sodium		Hemoglobin	
Potassium			
Glucose			
Uric acid			
CO_2			
Chloride			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; GGT, gamma-glutamyltransferase; IgG, immunoglobulin G; IgM, immunoglobulin M; NAb, neutralizing antibodies; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

According to the ICH definition, an AE (or adverse experience) is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the IP.”

An adverse drug reaction (ADR) is described by the ICH as “all noxious and unintended responses to a medicinal product related to any dose.” This means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, ie, the relationship cannot be ruled out.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The reporting period for non-serious AEs is the period from the first administration of study drug through the final F/U Visit or at the ETV. If a non-serious AE remains unresolved at the conclusion of the study, the PI and medical monitor will make a joint clinical assessment as to whether continued follow-up of the AE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

The Investigator will assess AEs for severity, for relationship to IP, and as to whether the event meets one or more of the definitions of an SAE (refer to Section 10.2).

The Investigator will determine the severity of each AE and will record it on the source documents and AE CRF, using the categories defined below.

Grade	Description
Mild	No limitation of usual activities
Moderate	Some limitation of usual activities
Severe	Inability to carry out usual activities

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the relationship of an AE to the IP and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	**Description**
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A SAE is defined as any AE that:

- Results in death.
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization.
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, that is, an AE that occurs in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Is an important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

More than one of the above criteria may apply to any specific event.

The reporting period for SAEs begins earlier than non-serious AEs and is the period from the time of signing of the ICF through 4 weeks after the last dose or at the ETV. SAEs reported to the Investigator outside of this reporting period will be reported to BioMarin if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

Any SAE, whether or not considered related to study drug, will be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate CRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying BioMarin Pharmacovigilance of an SAE. BioMarin may be required to

report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by BioMarin as soon as it becomes available.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history and demographics
- Physical examination and height
- Vital signs
- 12-lead ECG
- Clinical laboratory tests (including hematology, chemistry, and urinalysis)
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration

For subjects who participated in PAL-002, these assessments may be the same used for the Final Follow-up Visit of PAL-002, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done on the third day following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

12.3.2 Weekly Visits

The following study activities will be performed at the weekly visits beginning with Week 2:

- Vital signs
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasm tyrosine concentration
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.3 Monthly Visits (Week 4, 8, 16, etc)

The following study activities will be performed at the monthly visits beginning with Week 4:

- Vital signs
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

12.3.4 Quarterly Visits (Week 12, 24, 36, etc)

Quarterly visits consist of all monthly activities and include additional activities.
The following study activities will be performed at the quarterly visits beginning with Week 12:

- Physical examination
- Vital signs
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

12.4 Final Follow-up Visit (Day 113 [± 3 days]) /Early Termination Visit

The final follow-up (F/U) Visit or Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the F/U Visit or ETV:

- Physical examination and weight
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs and clinically significant changes in vital signs and laboratory test results.

The verbatim terms reported on CRFs to identify AEs will be coded using MedDRA. Treatment-emergent AEs will be summarized for each treatment group by system organ class, preferred term, relationship to study drug, and severity. Changes from baseline in vital signs and laboratory test results will be summarized with descriptive statistics by dose group.

14.3 Pharmacokinetic Analysis

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (below 480 µmol/L) for a minimum of 2 weeks and no further dose modification is planned.

Should data become available from PAL-001 and/or PAL-002 which indicate study drug accumulation should also be measured, the protocol will be amended to require additional blood draws for PK analysis.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any posttreatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled timepoint will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration

from baseline to each scheduled timepoint and presence/absence of antibodies will also be summarized.

14.5 Determination of Sample Size

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

At least one interim analysis may be performed by the sponsor during the study.

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act in an advisory capacity to BioMarin to monitor subject safety and the efficacy of rAvPAL-PEG in subjects who participate in the study. Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Study Medical Officer. The Chair will be notified by the Study Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study, on a schedule defined in the DMC Charter, and offer advice on whether or not to proceed, modify or terminate study enrollment on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.3.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the

clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of BioMarin, I confirm that BioMarin, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

[redacted]

2/18/09
Date

Printed name: [redacted]

BIOMARIN

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Study Title:	Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted] Clinical **Sciences** BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	**24 months** or until study is terminated
Dose:	0.001 to 1.0 mg/kg per individual injection, with a maximum weekly dose of 2.0 mg/kg
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	**October 30, 2009**

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 2

Date: October 30, 2009

RATIONALE AND SUMMARY OF CHANGES

1. The primary objective of the study has been revised for clarity; multiple doses of SC injections of rAvPAL-PEG will be assessed for efficacy on blood Phe levels of subjects with phenylketonuria (PKU).
2. Information regarding study drug dose adjustment (ie, allowable dose increases to achieve target Phe concentrations [ie, 120-600 µmol/L] and dose de-escalation for safety [ie, Phe concentration <120 µmol/L]) have been revised for clarity. Information regarding dose decreases relative to changes to subject diet has also been clarified.
3. Additional pharmacokinetic (PK) sampling has been added to allow for additional assessment of study drug exposure and accumulation based on PK information from the Phase 1 study, PAL-001 (A Phase 1, Open-Label, Dose-Escalation Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single, Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria).
4. The information regarding the 2 subjects who reported serious adverse events in Study PAL-001 (A Phase 1, Open-Label, Dose-Escalation Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single, Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria) has been updated. In addition, information regarding the future risk of using other PEG-containing or PEGylated injectable drugs has been added.
5. The Nonclinical Studies section has been updated to include information regarding chronic repeat-dose toxicity and toxicokinetic studies in monkeys and rats.
6. The stopping criteria have been revised for clarity.
    - The criterion for toxicity has been clarified; subjects who have a drug-related Common Terminology Criteria for Adverse Events (CTCAE) grade ≥ 3 event that is treatment emergent will stop study drug until resolution of the event.

- The criterion for stopping dosing within a dose level has been clarified; if 2 or more subjects within a dose level have a drug-related CTCAE grade ≥3 event that is treatment emergent, further dosing within that dose level will be stopped and no subjects in higher dose levels will be treated until a safety assessment is completed. The Food and Drug Administration (FDA) will also be notified.

7. Information regarding the management of local and systemic reactions to rAvPAL-PEG has been revised for clarity and to incorporate safety measures based on information from the Phase 1 study, PAL-001, and recommendations from an allergy advisory board.
    - The definition of local skin reactions (at or near the injection site) has been revised for further clarity.
    - Definitions of large local skin reactions that are contiguous and are not contiguous to the study drug injection site have been added.
    - The definition of systemic reactions has been revised to include skin (eg, generalized skin reaction) and non-skin symptoms; the definition has also been broadened for further clarity. In addition, the skin prick test for subjects with systemic reactions has been removed.
    - Information regarding subjects who have a systemic reaction that includes skin symptoms (eg, generalized skin reaction) has been revised. Subjects who have a generalized skin reaction will complete an Unscheduled Hypersensitivity Reaction Visit and will then be terminated from the study.
8. An Unscheduled Hypersensitivity Reaction Visit has been added for subjects who have a systemic reaction that includes skin symptoms (eg, generalized skin reaction) after administration of rAvPAL-PEG based on additional safety information from the Phase 1 study, PAL-001, and per the recommendation of an allergy advisory board.
    - The Unscheduled Hypersensitivity Reaction Visit includes clinical laboratory tests (urinalysis, hematology, chemistry) and tests for C-reactive protein (CRP), total hemolytic complement (CH50), complements C_1 and C_4, sedimentation rate, serum tryptase level, and serum anti-rAvPAL-PEG antibodies to be performed within 48 hours of the reaction. A PK sample will also be obtained.
    - Subjects who have a large local skin reaction that is not contiguous to the injection site and subjects who have a systemic reaction that does not include skin symptoms (generalized skin reaction) may also have the Unscheduled Hypersensitivity Reaction Visit assessments performed per Investigator discretion and in consultation with the Sponsor's Medical Officer.

- All subjects who complete an Unscheduled Hypersensitivity Reaction Visit will be terminated from the study and will complete the Early Termination Visit assessments.

9. Information regarding treatment of subjects with local skin reactions has been revised to incorporate recommendations made by an allergy advisory board.
    - For subjects who have had a prior local skin reaction to rAvPAL-PEG or PEG-containing product, pretreatment with oral steroids has been removed because long-term steroid use is not indicated for the study patient population. The preferred antihistamine has been changed from Claritin (loratadine) to Zyrtec (cetirizine).
    - Information regarding treatment of subjects who have a local skin reaction has been added. Subjects may be treated with non-sedating oral and/or topical antihistamines and/or topical steroids.
10. Safety, PK, and blood Phe concentration assessments have been added for subjects who have their dose of rAvPAL-PEG increased in frequency to more than 1x/week. Subjects who have study drug frequency increased to 2x/week or daily should perform the Interim Dosing Visit assessments.
11. Dosing information has been revised to incorporate the additional dose levels in PAL-002. In addition, information regarding self-administration of study drug by subjects has been removed; study drug will be administered by the study clinic staff or other qualified and trained study personnel only.
12. Information regarding missed doses has been added. Subjects who miss more than 2 scheduled doses will need to have their dose level and dose frequency re-evaluated.
13. Information regarding the number of enrolled subjects has been revised to incorporate changes to Study PAL-002. In addition, information regarding replacement of subjects who do not complete the study has been removed. Only subjects who complete Study PAL-002 are eligible for participation in this study.
14. Information regarding assessment for immunogenicity has been clarified; the specific antibody assessments have been clarified per FDA request.
15. A 3-day diet diary has been added to the weekly visits.
16. The severity grades for adverse event (AE) reporting have been revised; CTCAE grades 1-5 will now be used.
17. The study duration has been revised; subjects will complete the study after 24 months of treatment with study drug.
18. The target blood Phe concentration has been revised from 480 µmol/L to 120-600 µmol/L for consistency with Study PAL-002.

19. The efficacy analysis has been revised to include assessment of subjects who maintain a target Phe concentration. Subjects to be included in the safety analysis population have been revised for clarity; subjects who have postdose safety information will be included in the safety analysis.
20. PK, blood Phe, and tyrosine assessments have been revised for subjects who are on a stable dosing regimen for at least 3 months; subjects may have these assessments performed less frequently (ie, at the monthly versus weekly visits).
21. The timing of the Early Termination visit has been revised from 1 week to 4 weeks after the final dose of study drug to allow for an assessment that was recommended by an allergy advisory board prior to study termination.
22. Collection of subject's allergy history has been added at the Screening visit.
23. A chest X-ray has been added to the 12-month and 24-month visits.
24. Information regarding an Interactive Voice Response System (IVRS) has been removed; study drug assignment will not be conducted using an IVRS.
25. The Medical Monitor has been changed.
26. The Schedule of Events and the Study Procedure section (Section 12.3) have been updated to reflect the changes to this amendment. In addition, assessment schedules have been added for subjects who increase their dose frequency to 2x/week and daily (Interim Dosing Visit).
27. Administrative revisions have been made to improve clarity and consistency.

PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale. Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough~~ font.

Section No./Title	Revision	Comments	Relates to Change No.
Section 2/Synopsis (Objectives)	The primary objective of the study is: • To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU. The secondary objectives of the study are: • To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU ~~who participated in Study PAL-002~~.		1, 13, 27
Section 2/Synopsis (Study Design and Plan)	This is a long-term extension of a Phase 2, open-label, dose-finding study (Study PAL-002) in ~~approximately~~ 35-50 ~~35~~ subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002. Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety as determined by blood Phe concentrations. If a subject's blood Phe concentration is <120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.		2, 13, 18, 27
Section 2/Synopsis (Study Design and Plan)	At least one interim analysis may be performed by the sponsor during the study. In addition to the Sponsor, a~~A~~ Data Monitoring Committee (DMC) will monitor the ~~study~~ safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to		11, 17, 18, 27

Section No./Title	Revision	Comments	Relates to Change No.
	take rAvPAL-PEG. PAL-002 subjects' ~~participants~~' rAvPAL-PEG dosing will continue in PAL-003 without interruption of ~~weekly~~ dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of ~~below~~ 120-600~~480~~ µmol/L. ~~Subjects will continue to receive injections in the clinic, administered by clinic staff but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug d~~Doses will be evaluated on an individual basis. ~~The dose may be modified at any time for safety.~~ Evaluations, observations, and procedures will be conducted at selected time points ~~as shown in Table 2.1.1, Schedule of Events~~. After the subject's blood Phe concentration has been controlled to within a target range (~~below~~ 120-600~~480~~ µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed but not to exceed 3x/week (fingerstick tests may be performed more frequently). A subject will continue in PAL-003 until one of the following occurs: 1. The subject withdraws consent and discontinues from the study. 2. The subject is discontinued from the study at the discretion of the Investigator. ~~3. The drug becomes commercially available following the appropriate marketing approval.~~ The subject has completed the study through the Month 24 visit. 4. The study is terminated.		
Section 2/Synopsis (Study Design and Plan)	Dose Modifications: After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the subject for safety. Dose ~~Adjustment~~ Increase Methodology:		2, 11, 27

Section No./Title	Revision	Comments	Relates to Change No.
	Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on available blood Phe and drug concentrations. Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed. ~~Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made, so that blood Phe concentrations can be assessed~~. Depending upon the response to rAvPAL-PEG in PAL-002, an individual subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows. • The dose may be increased by increments of no more than 10-fold higher than the subject's current dose. • When ~~adjusting~~ increasing the dose, a dose may be used that is between the dose ~~cohort l~~evels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, and 1.0 mg/kg) that were defined in the previous study (PAL-002). • The dose may be adjusted by increasing the frequency up to daily ~~of~~~~number of injections per week, up to one dose per day,~~ for a ~~maximum~~ total weekly dose ~~per week of~~ not to exceed 2.0 mg/kg, including subjects who receive more than 1 dose/week. Subjects who have increased their dose frequency will have additional assessments performed (Interim Dosing Visit). • When a dose is ~~adjusted~~increased (either by increasing~~changing~~ the dose level or by increasing~~changing~~ the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time. • Only 1 dose adjustment is allowed every 2 weeks. • Blood Phe levels will be measured 3 days after each dose increase (fingerstick is		

Section No./Title	Revision	Comments	Relates to Change No.
	acceptable); more frequent or daily blood Phe measurements may be performed. • ~~The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration for safety.~~ Dose Decrease Methodology A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if blood Phe concentrations are <120 µmol/L. If a subject's blood Phe level is <120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is <120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (>30% change from baseline) and drug concentrations, as well as toxicity (any hypersensitivity reaction). Dose decreases may occur for safety (ie, blood Phe concentration <120 µmol/L) or other toxicity that may be improved with a lower, more frequent dose. A dose should first be reduced by dose level (1.0, 0.6, 0.3, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.		
Section 2/Synopsis (Study Design and Plan)	Stopping Criteria: If an individual subject exhibits ~~drug-related~~ toxicity of a treatment-emergent CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves.		6

Section No./Title	Revision	Comments	Relates to Change No.
	The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level. In addition, if 2 or more subjects at a dose level exhibit ~~drug related~~ toxicity of a treatment-emergent CTCAE grade 3 or greater, further dosing of subjects at that dose level will be halted and no subjects will be treated at a ~~any~~ higher dose level~~s will be halted~~ until a safety assessment is completed. In addition, the Food and Drug Administration (FDA) will be notified of this occurrence.		
Section 2/Synopsis (Number of Planned Subjects)	35-50~~35~~ subjects.		13, 27
Section 2/Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Numbering has been added to all of the criteria.		27
Section 2/Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera~~,within 6 months prior to Screening and~~ during study participation. 8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from ~~PAL-001 or~~ PAL-002.		27
Section 2/Synopsis	rAvPAL-PEG doses will be administered SC and the ~~favorable dose (the~~ dosage that provides control of blood Phe concentrations within the target range of 120-600 µmol/L		18, 27

Section No./Title	Revision	Comments	Relates to Change No.
(Investigational Product, Dose, Route and Regimen)	for a minimum of 2 weeks~~)~~ will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.		
Section 2/Synopsis (Duration of Treatment)	3. ~~The drug becomes commercially available following the appropriate marketing approval.~~ The subject has completed the study through the Month 24 visit.		17
Section 2/Synopsis (Criteria for Evaluation)	**Immunogenicity:** The presence of antibodies will be assessed.		27
Section 2/Synopsis (Safety Analysis)	All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.		19
Section 2/Synopsis (Efficacy Analysis)	Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 120-600 µmol/L will be summarized by each scheduled time point.		19
Section 2/Synopsis (Pharmacokinetic Analysis)	Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (~~below~~ 120-600~~480~~ µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. ~~Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.~~ Should data become available from PAL-002 that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed but not to exceed 3x/week.		3, 18
Section 2.1/Schedule of Events (Table 2.1:	The Schedule of Events has been revised to reflect changes to the study procedures. The Final F/U and Early Termination visits have been separated, and		8, 10, 15, 20, 21, 22, 23, 26

Section No./Title	Revision	Comments	Relates to Change No.
Schedule of Events)	an Unscheduled Hypersensitivity Reaction Visit has also been added. Tables 2.1.2 (Interim Dosing Visit Schedule, Subjects Whose Dose Is Increased to 2x/Week) and 2.1.3 (Interim Dosing Visit Schedule, Subjects Whose Dose Is Increased to Daily) have been added.		
Section 4/List of Abbreviations and Definition of Terms	CBC complete blood count CH50 total hemolytic complement C_{max} maximum plasma concentration CRP C-reactive protein CTCAE Common Termino~~al~~logy Criteria for Adverse Events ~~C_{trough} predose concentration of rAvPAL-PEG~~ ~~DCF Data Clarification Form~~ ~~IND Investigational New Drug~~ ~~IVRS interactive voice response system~~ IgE immunoglobulin E NOAEL no observable adverse effect level rAvPAL-PEG rAv phenylalanine ammonia lyase-PEG		27
Section 6/Investigators	The study will be administered by and monitored by employees or representatives		27

Section No./Title	Revision	Comments	Relates to Change No.
and Study Administrative Structure	of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.		
Section 7.1/Nonclinical Studies	Weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG (approximately 4 IU/mouse) for greater than 2 to 3 months lowered and stabilized plasma ~~blood~~ Phe concentration from approximately 2000 µM to less than 200 µM. The safety of rAvPAL-PEG was evaluated in safety pharmacology studies (respiratory, central nervous system [CNS] and cardiovascular [CV]) and toxicity with toxicokinetic studies (single and 28-day repeated dose) in rats and cynomolgus monkeys. Overall, in the nonclinical ~~in all toxicity~~ studies, no ~~immune related toxicities~~ anaphylactoid-like reactions or injection-site reactions were noted ~~were seen either systemically or at the injection site~~. No specific polyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies. The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized		5, 27

Section No./Title	Revision	Comments	Relates to Change No.
	muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups. No degeneration of the arteries was observed in either the control or animals administered ~~0 and~~ 0.01 mg/kg rAvPAL-PEG.		
Section 7.1/Nonclinical Studies	Chronic repeat-dose toxicity and toxicokinetic studies were conducted in the rat (17/26 weeks) and monkey (39 weeks). In the 26-week study of rats, weight loss was observed in the rats given 25 mg/kg/dose of rAvPAL-PEG, SC; corresponding decreased food consumption was observed in the males. The main histological finding was focal-to-multifocal areas of vacuolar degeneration of renal tubule cells in the kidney of 3 males given 25 mg/kg and 3 females given ≥8 mg/kg rAvPAL-PEG. This renal tubule finding persisted in the kidney of both sexes given ≥8 mg/kg/dose at the end of the 12-week recovery phase without evidence of reversibility. Increased vacuolation was observed in histiocytic cells of the liver, spleen, mesenteric lymph node, mandibular lymph node, and adrenal cortex of males given 25 mg/kg/dose and females given ≥8 mg/kg/dose; vacuolation of histiocytic cells in the testes were also observed in males given 25 mg/kg/dose. Both renal tubule vacuolation and histiocytic cell vacuoles have been associated with PEG-related catabolism and administration of other PEGylated proteins. In the 39-week, chronic repeat-dose toxicity and toxicokinetic study in monkeys, dose levels of 7.0 and 5.0 mg/kg/dose rAvPAL-PEG were not tolerated in female monkeys and resulted in body weight loss, decreased food consumption, and		5

Section No./Title	Revision	Comments	Relates to Change No.
	hypoactivity. The only adverse microscopic finding was arterial inflammation of small and mid-sized arteries in multiple organs and/or tissues in most animals that were given ≥3 mg/kg/dose rAvPAL-PEG SC twice weekly at the end of the dosing phase. The inter-individual organ involvement was highly variable; some animals had findings in one organ and others had multiple organs with arterial inflammation. Additionally, not all arteries had signs of inflammation and arterial inflammation was resolved by the end of the 13-week recovery period. A full analysis of these findings including the involvement of the anti-drug antibodies, antibody isotypes, PEG distribution, and rAvPAL-PEG is ongoing. The no observable adverse effect level (NOAEL) for the 17-week and 39-week studies, in rat and monkey, respectively, is 1 mg/kg/dose. The NOAEL from these studies represents greater than a 100-fold safety factor over the starting dose in PAL-002.		
Section 7.2/Previous Clinical Studies	This study is an extension of the second human clinical study with rAvPAL-PEG (Study PAL-002). Study PAL-001, the first-in-human clinical study of rAvPAL-PEG, was designed as a Phase 1, open-label, single-dose, dose-escalation study in ~~approximately 35~~25 subjects, 16 to 50 years old, with PKU. The doses for this study are based on data from nonclinical studies and PAL-001 and are~~ planned to be in the same range as those tested in PAL-001 and PAL-002 (~~0.001 through 1.0 mg/kg per injection (not to exceed 2.0 mg/kg/week~~)~~, provided no dose-limiting toxicity was observed in ~~those studies~~ Study PAL-002.		27
Section 7.4/Summary of Overall Risks and Benefits	It is not expected that data from ~~PAL-001 and~~ PAL-002 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant		4, 27

Section No./Title	Revision	Comments	Relates to Change No.
	PEGylated non-human enzymes currently in use.		
Section 7.4.1/Toxicity Due to Exposure to PEG	No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg ~~of SC~~ rAvPAL-PEG SC.		27
Section 7.4.2/Toxicity Due to an Immunologic Reaction	This section has been reorganized to include updated safety information from Study PAL-001. Presentation of information in this section, including section numbers, has also been revised.		27
Section 7.4.2/Toxicity Due to an Immunologic Reaction	The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. ~~In animal models, the immune response against the foreign protein PAL in rAvPAL-PEG has been mitigated by PEGylation.~~ Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. This may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.		4, 27
Section 7.4.2.1/ ~~Reactions with Depo-Provera~~Systemic Skin Reactions	~~Two subjects enrolled in PAL-001 who had received one dose of rAvPAL-PEG experienced hypersensitivity reactions to intramuscular (IM) Depo-Provera, a contraceptive containing PEG 3350. Both subjects had previously received multiple IM Depo-Provera injections without complications.~~ ~~The first subject, a [redacted] year old [redacted], developed bruising at the injection site on Day 1 that resolved on Day 15. [redacted] developed hives and mild respiratory~~		4, 27

Section No./Title	Revision	Comments	Relates to Change No.
	~~difficulty 10 minutes after receiving IM Depo-Provera given 40 days after [redacted] rAvPAL-PEG injection. [redacted] was treated in the emergency room with [redacted], [redacted], [redacted] d, and [redacted]; and oral [redacted] and [redacted]. The events resolved the same day, and the subject had no further complications. The subject had no prior history of any reactions to Depo-Provera. Subsequently, while under the care of an allergist/immunologist, the subject had a mild reaction to a concentrated intradermal (1:10) exposure of Depo-Provera. [redacted] was later given a full dose of IM Depo-Provera without problems.~~ ~~The second subject, an [redacted] year old [redacted], had a slightly raised, erythematic rash below the site of rAvPAL-PEG injection on Day 11 that resolved without treatment on Day 15. [redacted] developed generalized hives on [redacted] arms and legs and mild difficulty breathing 20 minutes after receiving IM Depo-Provera (and [redacted] third dose of Gardasil) given 15 days after [redacted] rAvPAL-PEG injection. [redacted] symptoms subsided after [redacted] received oral [redacted] given by [redacted] mother. [redacted] did not visit the emergency room. The subject had no prior history of any reactions to Depo-Provera or Gardasil (Gardasil dose not contain PEG). [redacted] was subsequently seen by an allergist/immunologist, and the results are pending.~~ ~~Because Depo-Provera contains PEG 3350 as an excipient, it is possible that the administration of rAvPAL-PEG caused anti-PEG antibody formation that led to a cross-reaction with Depo-Provera in these subjects. Therefore, it is possible that prior exposure to rAvPAL-PEG might sensitize subjects to other PEG-containing injectable drugs. Alternatively, the presence of Depo-Provera could have led to~~		

Section No./Title	Revision	Comments	Relates to Change No.
	~~antibody formation and cross-reaction with rAvPAL-PEG, leading to rAvPAL-PEG injection-site reactions. Since it is currently unknown whether Depo-Provera has a sensitizing effect to PEG and the exact basis for these reported events is not known, the use of PEG-containing injectable drugs prior to and during the study is prohibited as a precautionary measure (refer to the addendum and Sections 9.3.2 and 9.4.8).~~ Two subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. Both of these events were reported as serious and followed administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications. The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the [redacted]. Both subjects were examined by an allergy specialist after		

Section No./Title	Revision	Comments	Relates to Change No.
	they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. The subject continues to receive regular doses (every 3 months) of Depo-Provera, and no further reactions have been reported to date (15OCT2009). The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist. The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. It is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. ~~Since i~~It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because ~~and~~ the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to,		

Section No./Title	Revision	Comments	Relates to Change No.
	~~and~~ during, and after this study is prohibited as a precautionary measure (refer to Sections 9.3.2 and 9.4.8.).		
Section 7.4.2.2/ Management of Allergic Reactions	Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.1.1. Detailed instructions for the management of allergic reactions are provided in the Study Reference Manual.		27
Section 7.4.3/Effects of Low Blood Phe	If a subject's blood Phe concentration is too low, the subject's diet, current dose of study drug, or both may be adjusted.		1, 27
Section 8/Study Objectives	• To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU. The secondary objectives of the study are: • To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU ~~who participated in Study PAL-002~~.		1, 13, 27
Section 9.1/Overall Study Design and Plan	This is a long-term extension of a Phase 2, open-label, dose-finding study (PAL-002) in ~~approximately~~35-50 ~~35~~ subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002. Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety as determined by blood Phe concentrations. If a subject's blood Phe		2, 13, 27

Section No./Title	Revision	Comments	Relates to Change No.
	concentration is <120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. For information regarding changes to diet for safety and decreases in dose, refer to Section 9.1.2.2.		
Section 9.1/Overall Study Design and Plan	At least one interim analysis may be performed by the sponsor during the study. In addition to the Sponsor, a~~A~~ Data Monitoring Committee (DMC) will monitor the ~~study~~ safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 subjects' ~~participants~~' rAvPAL-PEG dosing will continue in PAL-003 without interruption of ~~weekly~~ dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations ~~below~~ of 120-600~~480~~ µmol/L ~~based upon recommendations by the German Working Group for Metabolic Diseases (Burgard, 1999, Eur J Pediatr~~). ~~Subjects will continue to receive injections in the clinic, administered by clinic staff., but may later be allowed to self-administer doses of study drug at home. Whether a subject may be allowed to self-administer study drug d~~Doses will be evaluated on an individual basis. ~~All of the following criteria must be met before a subject may self-administer doses of the study drug:~~ ~~The subject is willing and able to administer the study drug as directed.~~		11, 17, 18, 27

Section No./Title	Revision	Comments	Relates to Change No.
	~~The sponsor agrees that self-administration is appropriate.~~ ~~The subject has achieved a favorable dose (ie, the dosage that provides control of blood Phe concentrations within the target range for a minimum of 2 weeks).~~ ~~The dose may be modified at any time for safety.~~ Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been controlled to within a target range (~~below~~ 120-600~~480~~ µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed but not to exceed 3x/week (fingerstick tests may be performed more frequently). A subject will continue in PAL-003 until one of the following occurs: 1. The subject withdraws consent and discontinues from the study. 2. The subject is discontinued from the study at the discretion of the Investigator. ~~3. The drug becomes commercially available following the appropriate marketing approval.~~ The subject has completed the study through the Month 24 visit. 4. The study is terminated.		
Section 9.1.1/ Management of Local and Systemic Reactions to rAvPAL-PEG	Presentation of information in this section, including section numbers, has been revised.		27
Section	9.1.1.1 Subjects Who Have Had ~~Experienced~~ Previous Hypersensitivity		7-9

Section No./Title	Revision	Comments	Relates to Change No.
9.1.1/Management of Local and Systemic Reactions to rAvPAL-PEG	Reactions to rAvPAL-PEG or a PEG-Containing Product Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from ~~PAL-001 or~~ PAL-002 are excluded from participation in this study. Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in PAL-002 are eligible to participate in this study. ~~However, s~~Subjects may develop ~~such~~ systemic, large local skin, or local skin reactions after enrollment in PAL-003. ~~Management of such reactions described below. Refer to Figure 9 1.1.2.2.1 for a flowchart for managing these subjects.~~ Refer to Section 9.1.1.2 for definitions of local skin, large local skin, and systemic skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1. 9.1.1.2 Definition of Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows: Local skin reaction: • Skin signs or symptoms in 1 affected primary location, ~~1 affected location~~ ie, hives, wheals, or swelling or an area of erythema, ~~or~~ redness, induration, pain, or itching at or near the site of injection.		

Section No./Title	Revision	Comments	Relates to Change No.
	Large local skin reaction: • Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site. Systemic reaction (including generalized skin symptoms): • Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema, or ~~anaphylaxis or~~ the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.		
[redacted]	[redacted]		7-9, 27

Section No./Title	Revision	Comments	Relates to Change No.
	[redacted]		
9.1.1.3.1/Local Skin Reactions	~~After enrollment in PAL-003, s~~Subjects who have had a local skin reaction (refer to Section 9.1.1.2 for definition) after administration of~~to~~ rAvPAL-PEG in PAL-001, PAL-002, or this study ~~or a PEG-containing product will~~may be premedicated orally with acetaminophen and antihistamines ~~and/or oral steroids,~~ 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (~~Claritin~~ Zyrtec [cetirizine] is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg ~~Claritin~~ Zyrtec or 25 to 50 mg Benadryl [diphenahydramine]~~; an example for steroids would be 70 mg oral prednisone~~). Because Benadryl can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit. 9.1.1.3.2 Large Local Skin Reactions If a subject has a local skin reaction, a digital photograph of the reaction should be		7-9

Section No./Title	Revision	Comments	Relates to Change No.
	taken if possible. Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site. The location of the large local skin reaction relative to the rAvPAL-PEG injection site will determine if a subject may remain in the study and continue to be administered rAvPAL-PEG. An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk. Subjects who have large local skin reactions that are contiguous to the injection site may remain in the study. Subjects who have large local skin reactions that are not contiguous to the injection site may have additional assessments performed (Unscheduled Hypersensitivity Reaction Visit) per Investigator discretion and must then be terminated from the study. A decision to terminate a subject from the study must be agreed to by both the Investigator and the Sponsor's Medical Officer. 9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study. Large local skin reactions that are contiguous to the injection site should be managed as local skin reactions (refer to Section 9.1.1.3.1). For the remainder of the study, subjects must be premedicated		

Section No./Title	Revision	Comments	Relates to Change No.
	orally with acetaminophen and antihistamines 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (Zyrtec [cetirizine] is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Zyrtec or 25 to 50 mg Benadryl [diphenahydramine]). Because Benadryl can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver. Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2). 9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction. Subjects who develop large local skin reactions that are not contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may not continue to participate in the study. Subjects may have additional assessments performed (ie, Unscheduled Hypersensitivity Skin Reaction Visit) immediately after the reaction (within 48 hours): serum anti-rAvPAL-PEG antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL immunoglobulin M [IgM], anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG immunoglobulin E [IgE]); serum tryptase level;		

Section No./Title	Revision	Comments	Relates to Change No.
	sedimentation rate; C-reactive protein (CRP), total hemolytic complement (CH50), and complements C_1 and C_4; and clinical laboratory tests (urinalysis, chemistry, hematology). Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4). Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).		
Section 9.1.1.3.3/ Systemic Reactions	~~After enrollment in PAL-003, subjects who have a systemic reaction that includes noncutaneous systemic symptoms, such as respiratory problems, hypotension, or angioedema, to rAvPAL-PEG or a PEG-containing product will be evaluated with a skin prick test. If the test is positive, the subject will be excluded from the study.~~ ~~Subjects with prior systemic reactions that included only skin manifestations to rAvPAL-PEG or a PEG-containing product after PAL-003 enrollment will be premedicated with acetaminophen and antihistamines and/or oral steroids (one hour prior to study drug dosing) and will continue to receive rAvPAL-PEG.~~ ~~Reactions to skin prick testing or rAvPAL-PEG administered SC are defined as follows:~~ ~~Local reaction: 1 affected location, ie, hives, wheals, or swelling or an area of erythema or redness at or near the site of injection.~~		7, 8, 27

Section No./Title	Revision	Comments	Relates to Change No.
	~~Systemic reaction: more than 1 affected location, ie, cutaneous reaction in more than 1 area, and/or any other generalized symptoms, such as hypotension, angioedema or anaphylaxis or the involvement of other organ systems (eg, respiratory, cardiovascular, gastrointestinal).~~ Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG will be terminated from the study. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 48 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Skin Reaction Visit): serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE); serum tryptase level (optional; it is recommended that this sample be drawn immediately after reaction); sedimentation rate; CRP, CH50, C_1, and C_4; and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1). Subjects who have a systemic reaction without generalized skin symptoms may have these assessments performed per Investigator discretion. Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4).		
Section 9.1.2/Dose Modifications	After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the		2, 27

Section No./Title	Revision	Comments	Relates to Change No.
	subject for safety.		
Section 9.1.2.1/Dose Increase Methodology	Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on available blood Phe and drug concentrations. Depending upon the response to rAvPAL-PEG in PAL-002, an individual subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows: • The dose may be increased by increments of no more than 10-fold higher than the subject's current dose. • When ~~adjusting~~ increasing the dose, a dose may be used that is between the dose ~~cohort l~~evels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, and 1.0 mg/kg) that were defined in the previous study (PAL-002). • The dose may be adjusted by increasing the frequency up to daily ~~of number of injections per week, up to one dose per day,~~ for a ~~maximum~~ total weekly dose ~~per week of~~ not to exceed 2.0 mg/kg, including subjects who receive more than 1 dose/week. Subjects who increase their dose frequency should perform the Interim Dosing Visit assessments (refer to Section 12.3.5 and Tables 2.1.2 and 2.1.3). • When a dose is ~~adjusted~~increased (either by increasing~~changing~~ the dose level or by increasing~~changing~~ the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time. • Only 1 dose adjustment is allowed every 2 weeks.		2, 11, 27

Section No./Title	Revision	Comments	Relates to Change No.
	• Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed. • ~~The dose may be decreased as appropriate to achieve or maintain a favorable blood Phe concentration for safety.~~ ~~Subjects must have blood Phe concentration tested 3 days after a dose adjustment. There must be a minimum of 2 weeks after each dose adjustment before any additional dose adjustments may be made, so that blood Phe concentrations can be assessed~~. 9.1.2.2 Dose Decrease Methodology A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if blood Phe concentrations are <120 µmol/L. If a subject's blood Phe level is <120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is <120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (>30% change from baseline) and		

Section No./Title	Revision	Comments	Relates to Change No.
	drug concentrations, as well as toxicity (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for safety (ie, blood Phe concentration <120 µmol/L) or other toxicity that may be improved with a lower, more frequent dose. A dose should first be reduced by dose level (1.0, 0.6, 0.3, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.		
Section 9.1.3/Stopping Criteria	If an individual subject exhibits ~~drug related~~ toxicity of a treatment-emergent CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level. In addition, if 2 or more subjects at a dose level exhibit ~~drug related~~ toxicity of a treatment-emergent CTCAE grade 3 or greater, further dosing of subjects at that dose level will be halted and no subject will be treated at a ~~any~~ higher dose level~~s will be halted~~ until a safety assessment is completed. In addition, the Food and Drug Administration (FDA) will be notified of this occurrence.		6
Section 9.3.2/Exclusion Criteria	3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, ~~within 6 months prior to Screening and~~		27

Section No./Title	Revision	Comments	Relates to Change No.
	during study participation. 8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002.		
Section 9.3.4/Subject Identification and Replacement of Subjects	~~After enrollment of 35 subjects, if any single cohort (identified during PAL-002) has less than 3 subjects due to early terminations, those subjects will be replaced. Replacement subjects will enter the study at the same dose cohort level as the subject they are replacing.~~ ~~Each subject was assigned a unique subject identifier in either PAL-001 or PAL-002~~. Subjects will retain the same subject number assigned ~~used~~ in PAL-002. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used. Subjects who do not complete the study will not be replaced. ~~This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.~~		13, 27
Section 9.4.4/Directions for Administration	Study drug will be administered by clinic staff or other qualified and trained study personnel. **Table 9.4.4.1 Number of Injections Required For an Individual Weighing 80 kg**		11, 27

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td></td><td>
<table>
<tr><th>Dose Group (mg/kg)</th><th>Weight (kg)</th><th>Volume of Study Drug (mL)[a]</th><th>No. of Injections[b]</th></tr>
<tr><td>0.001</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.003</td><td>80</td><td>0.6</td><td>1</td></tr>
<tr><td>0.01</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.03</td><td>80</td><td>0.2</td><td>1</td></tr>
<tr><td><u>0.06</u></td><td><u>80</u></td><td><u>0.5</u></td><td><u>1</u></td></tr>
<tr><td>0.1</td><td>80</td><td>0.8</td><td>1</td></tr>
<tr><td>0.3</td><td>80</td><td>2.4</td><td>2</td></tr>
<tr><td><u>0.6</u></td><td><u>80</u></td><td><u>4.8</u></td><td><u>2 or 3</u></td></tr>
<tr><td>1.0</td><td>80</td><td>8.0</td><td>4</td></tr>
</table>
<u>Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency will need to be re-evaluated.</u>
</td><td></td><td></td></tr>
<tr><td>Section 9.4.5/Method of Assigning Subjects to Treatment Groups</td><td>~~An interactive voice response system (IVRS) will be used to track subjects' assigned dose and study drug inventory.~~</td><td></td><td>24</td></tr>
<tr><td>Section 9.4.6/Selection of Doses Used in the Study</td><td>This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A ~~favorable~~ dose that produces a reduction in blood Phe concentrations to ~~below~~ <u>120-600</u>~~480~~ µmol/L will be determined for each subject.</td><td></td><td>18, 27</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
	The doses for this study are based upon the results of nonclinical studies and PAL-001. ~~Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included severe morbidity, decreased plasma Phe concentration, decreased protein synthesis, and GI lesions. Plasma Phe concentrations were reduced to below the level of detection in the lower dose groups, without other findings (refer to Section 7.1)~~. The no observable adverse effect level (NOAELs) from nonclinical studies in rats and monkeys represents a greater than 100-fold safety factor over the starting dose administered in PAL-002, (0.001 mg/kg). ~~The doses for this study will be based upon the results of PAL-001 and PAL-002.~~		
Section 9.4.6.1/Selection of Timing of Dose for Each Subject	Study drug will be administered in the morning by clinic staff or other qualified and trained study personnel ~~or by the subject, if appropriate~~.		11, 27
Section 9.4.8/Prior and Concomitant Medications	Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines ~~and/or oral steroids~~ (refer to Section 9.1.1). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be		7-9, 27

Section No./Title	Revision	Comments	Relates to Change No.
	withdrawn from the study and complete an Early Termination Visit. Subjects who have had a prior systemic ~~hypersensitivity~~ reaction to rAvPAL-PEG or a PEG-containing product are excluded from participation in this study ~~will be premedicated with acetaminophen and antihistamines and/or oral steroids~~ (refer to Section 9.1.1). ~~If the local or systemic reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study.~~		
Section 9.6/Dietary or Other Protocol Restrictions	Subjects will be instructed that diet should not be altered during the course of the study except as necessary for safety. If a subject's blood Phe concentration is <120 µmol/L, the subject's diet will be adjusted to maintain blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next dose of study drug. The dietary record will be maintained with the study source documents.		2, 15, 27

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td>Section 9.7.1/Efficacy and Safety Measurements Assessed</td><td>
Table 9.7.1.1: Summary of Laboratory Assessments
<table>
<tr><th>Laboratory Assessment</th><th>Party Responsible for Performing Test</th><th>Section in Protocol for Details</th></tr>
<tr><td>Clinical laboratory tests</td><td>Central laboratory</td><td>9.7.5</td></tr>
<tr><td>Antibody testing</td><td>BioMarin[a]</td><td>9.7.4.2</td></tr>
<tr><td>PK variables</td><td>~~Central laboratory~~BioMarin</td><td>9.7.3</td></tr>
<tr><td>Blood Phe concentrations</td><td>Central laboratory</td><td>9.7.2.1</td></tr>
<tr><td>Urinalysis, pregnancy test (urine), and sedimentation rate</td><td>Local laboratory</td><td>9.7.5, 9.7.4.1</td></tr>
</table>
Phe, phenylalanine; PK, pharmacokinetic.
[a]Analysis of the anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.
</td><td></td><td>27</td></tr>
<tr><td>Section 9.7.1.1/ Demographic Data and Medical History</td><td>Demographic data and a detailed medical history, including allergy history, will be obtained at Screening.</td><td></td><td>22</td></tr>
<tr><td>Section 9.7.1.2/ Physical Examination Findings</td><td>Physical examination will include assessment of ~~weight;~~ general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. ~~Height will be measured at~~</td><td></td><td>27</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
	~~Screening only.~~		
Section 9.7.1.3/Vital Sign Measurements	Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, weight (kg), and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only.		27
Section 9.7.1.5/Chest X-Ray	A chest X-ray will be performed at the Month 12 and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.		23
Section 9.7.2.1/Blood Phenylalanine Concentrations	In addition, ~~3 days~~ after each administration of study drug, the subject will have a blood Phe measurement by fingerstick as outlined in Table 2.1.1. This may be done by the subject at home. Subjects who have their dose frequency increased will have additional Phe assessments performed as outlined in Table 2.1.2 and 2.1.3.		27
Section 9.7.3/ Pharmacokinetic Variables	Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentrations (~~below~~ 120-600~~480~~ µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks. Subjects who have increased their dose frequency to twice weekly or daily will		10, 18

Section No./Title	Revision	Comments	Relates to Change No.
	have additional PK assessments performed for 5 weeks after the change in dose regimen (refer to Section 12.3.5 and Tables 2.1.2 and 2.1.3).		
Section 9.7.4.1/ Pregnancy Testing	Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 2.1.1 and Table 2.1.2). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.		27
Section 9.7.4.2/ Antibody Testing	~~Immunoglobulin G (IgG) and M (IgM) antibodies and neutralizing antibodies (NAb) to rAvPAL-PEG will be measured using validated immunogeneticity assays at the timepoints indicated in the Schedule of Events (Table 2.1.1). Anti-PEG antibodies will also be measured.~~ Immunogenicity will be assessed by determining antibody response and neutralizing activity with the following measurements: 1) anti-rAvPAL immunoglobulin G (IgG) antibodies, 2) anti-rAvPAL immunoglobulin M (IgM) antibodies, 3) anti-PEG IgG antibodies, 4) anti-PEG IgM antibodies, 5) anti-rAvPAL-PEG immunoglobulin E (IgE) antibodies, and 6) anti-rAvPAL-PEG Enzyme Activity Neutralizing antibodies (NAb). Validated immunogenicity assays will be used per the time points indicated in the Schedule of Events (Table 2.1.1). BioMarin will perform the analysis except for anti-rAvPAL-PEG IgE antibodies, which will be assessed by a Contract Research Organization.		14

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td>Section 9.7.5/Clinical Laboratory Assessments</td><td>Table 9.7.5.1: Clinical Laboratory Tests has been revised to include tests for the Unscheduled Skin Reaction visits. In addition, information has been added to indicate that a local laboratory will perform sedimentation rate analysis and the other laboratory tests have been revised for accuracy.</td><td></td><td>8, 27</td></tr>
<tr><td>Section 10.1/Adverse Events</td><td>The Investigator will determine the severity of each AE and will record it on the source documents and AE CRF, using the ~~categories~~ CTCAE v 3 grades defined below. Events that are CTCAE grades 4 and 5 are serious events and require completion of both an SAE form and AE eCRF.

<table><tr><th>Grade</th><th>Description</th></tr><tr><td>1</td><td>Mild</td></tr><tr><td>2</td><td>Moderate</td></tr><tr><td>3</td><td>Severe</td></tr><tr><td>4</td><td>Life threatening or debilitating</td></tr><tr><td>5</td><td>Death related</td></tr><tr><td>~~Mild~~</td><td>~~No limitation of usual activities~~</td></tr><tr><td>~~Moderate~~</td><td>~~Some limitation of usual activities~~</td></tr><tr><td>~~Severe~~</td><td>~~Inability to carry out usual activities~~</td></tr></table></td><td></td><td>16</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
Section 10.4/BioMarin Pharmacovigilance Contact Information	Contact information for the Mmedical Mmonitor is as follows: Name: [redacted], MD Address: 105 Digital Drive Novato, CA 94949 USA Phone: [redacted] Fax: [redacted] E-mail: [redacted]		25
Section 12.2/Screening Visit	• Medical history, including allergy history, and demographics • Physical examination and height • Vital signs, including height and weight • Predose bBlood Phe and plasma tyrosine concentration For subjects who participated in PAL-002, these assessments may be the same used for the Week 16 Final Follow up Visit of PAL-002, if they occur within 28 days from Day 1.		22, 27
Section 12.3.1/Day 1 (Week 1)	• Vital signs, including weight • Predose PK sample • Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)		27
Section 12.3.2/Weekly	• Vital signs, including weight		15, 20, 27

Section No./Title	Revision	Comments	Relates to Change No.

Visits	• 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)
	• Predose PK sample
	  ○ Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.
	• Predose blood Phe and plasma tyrosine concentration
	  ○ Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.		

Section 12.3.3/Monthly Visits	Monthly visits consist of all weekly activities and include additional activities. The following study activities will be performed at the monthly visits beginning with Week 4: • Vital signs, including weight		15, 20, 27

Section No./Title	Revision	Comments	Relates to Change No.

	• Chest x-ray (Month 12 visit only)
	• 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)
	• Predose PK sample (subjects who are on a stable dosing regimen for ≥3 months only)
	  ○ Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.
	• Predose blood Phe and plasma tyrosine concentration
	  ○ Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.
	• Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)		

Section No./Title	Revision	Comments	Relates to Change No.
	In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.		
Section 12.3.4/Quarterly Visits (Week 12, 24, 36, etc.)	Quarterly visits consist of all weekly and monthly activities and include additional activities. The following study activities will be performed at the quarterly visits beginning with Week 12: • Vital signs, including weight • 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.) • Predose PK sample (subjects who are on a stable dosing regimen for ≥3 months only) o Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes. • Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE) In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.		15, 20, 27

Section No./Title	Revision	Comments	Relates to Change No.
Section 12.3.5/Interim Dosing Visit	Subjects who increase their dose frequency from 1x/week to 2x/week or daily will have additional assessments performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased. If the Interim Dosing Visit coincides with a weekly, monthly, or quarterly scheduled visit, the scheduled visit assessments should also be performed. The following study activities will be performed for the Interim Dosing Visit: • Vital signs, including weight • Injection-site inspection (previous and current injection site) • Assessment of AEs • Concomitant medications • Blood Phe measurement by fingerstick o This may be done by the subject at home. • PK sample o Subjects who increase their dose frequency to 2x/week will have an additional predose PK sample obtained prior to administration of the first 4 doses (ie, for 2 weeks after the dose frequency increase). For the remaining 3 weeks after the dose frequency increase, a PK sample will be obtained weekly prior to the first dose. Refer to Table 2.1.2 for the Interim Dosing Visit schedule. o Subjects who have their dose frequency increased to daily will have additional PK samples obtained every day for the first week: 12 hours after the first daily dose and predose every day for the		10

Section No./Title	Revision	Comments	Relates to Change No.
	rest of the week. After the first week of daily dosing, subjects will have weekly PK samples obtained for 4 additional weeks. Refer to Table 2.1.3 for the Interim Dosing Visit schedule.		
Section 12.3.6/ Unscheduled Hypersensitivity Reaction Visit	Subjects who have a generalized skin reaction after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 48 hours) the reaction. Subjects who have other systemic symptoms or a large local skin reaction that is not contiguous to the injection site (refer to Section 9.1.1) may have these assessments performed per investigator discretion. • Injection-site inspection • Clinical laboratory tests • Sedimentation rate • CRP, CH50, C_1, and C_4 • Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE) • Serum tryptase level o Perform immediately after reaction if possible. o Assessment is optional for subjects who have a generalized skin reaction per investigator discretion. • PK sample		8

Section No./Title	Revision	Comments	Relates to Change No.
	• Assessment of AEs • Concomitant medications Subjects should then return to the clinic 4 weeks after their last dose of rAvPAL-PEG for the Early Termination Visit assessments (refer to Section 12.4).		
Section 12.4/Early Termination Visit	12.4 ~~Final Follow-up Visit (Day 113 [±3 days])~~/Early Termination Visit The ~~final follow-up (F/U) Visit or~~ Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who complete an Unscheduled Hypersensitivity Reaction Visit (refer to Sections 12.3.6) will be terminated from the study. Subjects will then return 4 weeks after the last dose of study drug for the Early Termination Visit. The following study activities will be performed at the ~~F/U Visit or~~ ETV: • Physical examination ~~and weight~~ • Vital signs, including weight • Chest x-ray • PK sample • ~~Predose b~~Blood Phe and plasma tyrosine concentration • Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-		21, 27

Section No./Title	Revision	Comments	Relates to Change No.
	rAvPAL-PEG IgE)		
Section 12.5/Final Follow-up Visit ~~(Day 113 [±3 days])~~	12.54 Final Follow-up Visit (~~Day 113 [±3 days]~~) ~~/Early Termination Visit~~ ~~Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.~~ ~~Every reasonable effort should be made to contact any subject who is lost to follow up.~~ The following study activities will be performed at the F/U Visit ~~or ETV~~: • Vital signs, including weight • Chest x-ray • PK sample • ~~Predose b~~Blood Phe and plasma tyrosine concentration • Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)		21, 27
Section 14.2/Safety Analysis	All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses. Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.		19, 27

Section No./Title	Revision	Comments	Relates to Change No.
	The verbatim terms reported on CRFs to identify AEs will be coded using MedDRA. Treatment-emergent AEs will be summarized ~~for each treatment group~~ by system organ class, preferred term, relationship to study drug, and severity. Changes from baseline in vital signs and laboratory test results will be summarized with descriptive statistics ~~by dose group~~.		
Section 14.3/ Pharmacokinetic Analysis	Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (~~below~~ 120-600~~480~~ µmol/L) for a minimum of 2 weeks and no further dose modification is planned. Should data become available from ~~PAL-001 and/or~~ PAL-002 ~~which~~ that indicate study drug accumulation should also be measured, ~~the protocol will be amended to require~~ additional blood draws may be added for PK analysis as needed but not to exceed 3x/week.		18, 27
Section 14.4/Efficacy Analysis	In addition, the proportion of subjects who have maintained target Phe concentrations of 120-600 µmol/L will be summarized by each scheduled time point.		19
Section 14.7/Interim Analysis	At least one interim analysis may be performed by the sponsor during the study. Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).		27
Section 15/Data Monitoring Committee	The DMC will act independently in an advisory capacity to BioMarin to monitor		27

Section No./Title	Revision	Comments	Relates to Change No.
(DMC)	the ~~subject~~safety ~~and the efficacy~~ of rAvPAL-PEG in subjects who participate in the study.		
Section 21/References	~~Burgard, Bremer, Bührdel, Clemens, Mönch, Przyrembel, Trefz, Ullrich. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. Eur J Pediatr 158, 46-54. 1999.~~ ~~Butterworth. Effects of hyperammonaemia on brain function. J Inherit Metab Dis 21 Suppl 1, 6-20. 1998.~~		27

BIOMARIN

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical **Sciences** BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	**24 months** or until study is terminated
Dose:	0.001 to 1.0 mg/kg per individual injection, with a maximum weekly dose of 2.0 mg/kg
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	**October 30, 2009**

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 8 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (Study PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of **multiple doses of** SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

STUDY DESIGN AND PLAN:

This is a long-term extension of a Phase 2, open-label, dose-finding study (Study PAL-002) in 35-**50** subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety **as determined by blood Phe concentrations. If a subject's blood Phe concentration is <120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.**

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

At least one interim analysis may be performed by the sponsor during the study. In addition to the Sponsor, a Data Monitoring Committee **(DMC)** will monitor the **safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor**.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 **subjects'** rAvPAL-PEG dosing will continue in PAL-003 without interruption of dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations **of 120-600** µmol/L. **D**oses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (**120-600** µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses **as needed but not to exceed 3x/week (fingerstick tests may be performed more frequently)**.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. **The subject has completed the study through the Month 24 visit.**
4. The study is terminated.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

Dose Modifications:

After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the subject for safety.

Dose **Increase** Methodology:

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. **Decisions regarding dose increases will depend on blood Phe and drug concentrations**.

Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.

Depending upon the response to rAvPAL-PEG in PAL-002, **an individual** subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When **increasing** the dose, a dose may be used that is between the dose levels **(0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, and 1.0 mg/kg)** that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency **up to daily** for **a total weekly dose not to exceed** 2.0 mg/kg, **including subjects who receive more than 1 dose/week**. **Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit).**
- When a dose is **increased** (either by **increasing** the dose level or by **increasing** the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.
- **Only 1 dose adjustment is allowed every 2 weeks.**
- **Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.**

Dose Decrease Methodology

A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if a subject's blood Phe concentrations <120 µmol/L. If a subject's blood Phe level is

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

<120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is <120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (>30% change from baseline) and drug concentrations, as well as toxicity (any hypersensitivity reaction). Dose decreases may occur for safety (ie, blood Phe concentration <120 µmol/L) or other toxicity that may be improved with a lower, more frequent dose.

A dose should first be reduced by dose level (1.0, 0.6, 0.3, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

Stopping Criteria:

If an individual subject exhibits toxicity of **a treatment-emergent** CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit toxicity of **a treatment-emergent** CTCAE grade 3 or greater, further dosing of subjects at that dose level **will be halted** and **no subjects will be treated at a** higher dose level until a safety assessment is completed. **In addition, the Food and Drug Administration (FDA) will be notified of this occurrence.**

NUMBER OF SUBJECTS PLANNED:

35-**50** subjects.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in PAL-002.
2. Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, during study participation.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

rAvPAL-PEG doses will be administered SC and the dosage that provides control of blood Phe concentrations within the target range **of 120-600 µmol/L** for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.

DURATION OF TREATMENT:

Extension of multiple dosing will continue until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. **The subject has completed the study through the Month 24 visit.**
4. The study is terminated.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:
None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

The presence of antibodies will be assessed.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs or laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured when steady-state levels of Phe are attained.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

Safety Analysis:

All subjects who receive any amount of study drug **in this study and have postdose safety information** will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point **and presence/absence of antibodies** will also be summarized. **In addition, the proportion of subjects who have maintained target Phe**

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

concentrations of 120-600 µmol/L will be summarized by each scheduled time point.

Pharmacokinetic Analysis:

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (**120-600** µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. **Should data become available from PAL-002 that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed but not to exceed 3x/week.**

2.1 Schedule of Events

Table 2.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]				Final F/U Visit	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours; then perform Early Term. procedures
		Week 1	Weekly	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)			
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	1 week after final dose	4 weeks after final dose	
Informed consent	X							
Medical history**, including allergy history,** and demographics	X							
Physical examination[e]	X	X			X	X	X	
Vital signs **and weight**	X	X	X			X	X	
12-lead ECG	X					X	X	
Clinical laboratory tests[f]	X			X		X	X	**X**[g]
Sedimentation rate		X			X	X	X	**X**
Chest x-ray				**X (12-month visit only)**		**X**	**X**	
Urine pregnancy test[h]	X	X		X		X	X	
Injection-site inspection[i]		X (postdose)	X			X	X	**X**
Adverse events[j]	X←----------					----------→X	X	**X**
Concomitant medications	X	X	X			X	X	**X**
Diet query	X	X	X			X	X	
3-Day diet diary[k]			**X**					
Serum rAvPAL-PEG antibodies[l]		X		X		X	X	**X**
Predose **plasma** Phe and plasma tyrosine[m]	X	X	X[n]			X **(postdose)**	**X (postdose)**	
Plasma Phe (fingerstick)[o]			(3 days postdose)					
Plasma PK sample[p]		**X**	**X**[n]			**X**	**X**	**X**
Administer IP[q]		X	X					
Serum tryptase level[r]								**X**

AE, adverse event; **CH50, total hemolytic complement; CRP, C-reactive protein;** D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; **Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M;** IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] It is preferable to perform PAL-003 Screening assessments the same day as PAL-002 **Week 16 visit** so there is no interruption of study drug. If performed on the same day, PAL-002 **Week 16 visit** data may be used. If not, PAL-003 Day 1 must occur ≤ 28 days after PAL-003 Screening.

[b] Additional visits may occur if deemed necessary to monitor AEs or blood Phe, adjust dosing etc.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] **Unscheduled hypersensitivity reaction assessments are mandatory for subjects who have a generalized skin reaction. Subjects who have other systemic symptoms or who have a large local skin reaction that is not contiguous to the injection site may have these assessments performed per investigator discretion. Assessments should be performed within 48 hours of the reaction. Subjects who complete an Unscheduled Hypersensitivity Reaction Visit must be terminated from the study and should complete an Early Termination Visit.**

[e] Complete physical examinations to include the evaluation of all major body systems. **Height will be measured at Screening only**.

[f] Clinical laboratory tests to include hematology, chemistry, and urinalysis.

[g] **Subjects who have a generalized skin reaction should also be assessed for CRP, CH50, C_1, and C_4 within 48 hours of the reaction. Subjects who have other systemic symptoms or who have a large local skin reaction that is not contiguous to the injection site may have these assessments performed per investigator discretion.**

[h] **If positive or equivocal, perform serum pregnancy test.**

[i] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, e.g. right arm vs. left arm).

[j] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[k] **It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.**

[l] **Includes testing for anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE antibodies.**

[m] Samples should be drawn in the morning, at least 2.5 hours after a meal. **If dose is modified by increasing dose level, additional blood Phe concentration testing should be done on the third day following dose adjustment.** If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

[n] **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe, tyrosine, and PK assessments may be skipped at the weekly visits and performed at the monthly visits for**

the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced by a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.

[o] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

[p] The PK blood samples will be collected after the subject has achieved and maintained target blood Phe concentration**s** (**120-600** µmol/L) for a minimum of 2 weeks. Once this target is achieved, predose PK samples will be collected on the same day each week for 3 consecutive weeks.

[q] Dosing is up to 1.0 mg/kg per injection and up to one dose per day, for a total **not to exceed** 2.0 mg/kg/week, **including subjects who receive more than 1 dose/week**. **Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency will need to be re-evaluated.**

[r] **Mandatory for subjects who have a large local skin reaction that is not contiguous to the injection site or non-skin systemic symptoms. Optional for subjects who have a generalized skin reaction. Take sample immediately after reaction if possible.**

Table 2.1.2: Interim Dosing Visit Schedule, Subjects Whose Dose is Increased to 2x/Week

Assessments and Events	Subjects Who Are Administered rAvPAL-PEG 2x/Week Treatment Period[a, b]									
	First Week After Dose Adjustment		Second Week After Dose Adjustment		Third Week After Dose Adjustment		Fourth Week After Dose Adjustment		Fifth Week After Dose Adjustment	
	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose
Vital signs and weight	X	X	X	X	X	X	X	X	X	X
Injection-site inspection	X	X	X	X	X	X	X	X	X	X
Adverse events[c]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Plasma PK sample	X	X	X	X	X		X		X	
Blood Phe (fingerstick)[d]	X	X	X	X	X	X	X	X	X	X

AE, adverse event; D, day; PK, pharmacokinetics; Phe, phenylalanine.

[a] The additional Interim Dosing Visit assessments should be performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased to 2x/week. All assessments should be performed predose unless specified otherwise.

[b] If the Interim Dosing Visit coincides with a regularly scheduled visit, the scheduled visit assessments should also be performed.

[c] If any injection site reaction is observed, the corresponding AE data must specify injection location (identify current vs. previous visit's injection, e.g,. right arm vs. left arm).

[d] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

Table 2.1.3: Interim Dosing Visit Schedule, Subjects Whose Dose is Increased to Daily

Assessments and Events	Subjects Who Are Administered rAvPAL-PEG Daily Treatment Period[a, b]					
	First Day After Dose Adjustment	First Week After Dose Adjustment	Second Week After Dose Adjustment	Third Week After Dose Adjustment	Fourth Week After Dose Adjustment	Fifth Week After Dose Adjustment
	Dose 1	Doses 2-7	Doses 8-14	Doses 15-21	Doses 22-29	Doses 30-36
Vital signs and weight	X	X	X	X	X	X
Injection-site inspection	X	X	X	X	X	X
Adverse events[c]	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X
Plasma PK sample	X (12 hours postdose)	X (Daily)	1x/week	1x/week	1x/week	1x/week
Blood Phe (fingerstick)[d]	X	X	X	X	X	X

AE, adverse event; D, day; PK, pharmacokinetics; Phe, phenylalanine.

[a] The additional Interim Dosing Visit assessments should be performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased to daily. All assessments should be performed predose unless specified otherwise.

[b] the Interim Dosing Visit coincides with a regularly scheduled visit, the scheduled visit assessments should also be performed.

[c] If any injection site reaction is observed, the corresponding AE data must specify injection location (identify current vs. previous visit's injection, e.g,. right arm vs. left arm).

[d] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

[redacted]

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
CBC	**complete blood count**
CD	Compact disk
CFR	Code of Federal Regulations
CH50	**total hemolytic complement**
C_{max}	**maximum plasma concentration**
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	**C-reactive protein**
CTCAE	Common Termin**ology** Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal

IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	**immunoglobulin E**
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
NOAEL	**no observable adverse effect level**
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	**rAv** phenylalanine ammonia lyase**-PEG**
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation

SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR §50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. **Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.**

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The murine model of phenylketonuria (PKU) BTBR*Pah*^{enu2} (ENU2), a mouse line that is deficient in phenylalanine hydroxylase (PAH) activity (Shedlovsky, 1993, Genetics), was used for in vivo screening and pharmacodynamic studies. This mouse model exhibits characteristics similar to those seen in PKU patients, including hyperphenylalaninemia (baseline plasma phenylalanine [Phe] concentrations of 1000 to 2000 µM) and hypopigmentation. Weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG (approximately 4 IU/mouse) for greater than **2 to** 3 months lowered **and stabilized plasma** Phe concentration from approximately 2000 µM to less than 200 µM. A similar profile of Phe reduction was also seen in ENU2 mice administered wild-type AvPAL-PEG (4 IU/mouse) over 8 weeks. In all these studies, regardless of the molecule, an attenuated Phe-lowering response was usually seen between Weeks 2 and 7. However, plasma Phe concentrations became stable at concentrations below 200 µM from Week 7 onward. In addition to the reduction of plasma Phe, a dose-related darkening in coat color occurred, indicating the biosynthesis of melanin, which was previously inhibited when plasma Phe could not be metabolized and plasma Phe concentrations were high. When administration of AvPAL-PEG was stopped for 2 to 7 weeks and then reinitiated with weekly administrations, no attenuated response was observed and Phe concentrations remained low and stable until the next administration.

The safety of rAvPAL-PEG was evaluated in safety pharmacology studies (respiratory, central nervous system [CNS] and cardiovascular [CV]) and toxicity with toxicokinetic studies (single and 28-day repeated dose) in rats and cynomolgus monkeys. Overall, in **the nonclinical** studies, no **anaphylactoid-like reactions or injection-site reactions were noted.** No specific polyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies.

Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included reduced food consumption, dehydration, body weight loss, hypoactivity, hypothermia,decreased plasma Phe concentration, decreased protein synthesis, and gastrointestinal (GI) lesions. Plasma Phe concentrations were also reduced to below the

level of detection in the 4 and 12 mg/kg dose groups, but without similar toxicological consequences, implying that the physiological regulation of Phe levels may be an important factor for influencing morbidity.

The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups. No degeneration of the arteries was observed in **either the control or** animals administered 0.01 mg/kg rAvPAL-PEG.

The half-life of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg, was 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose linearity. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the half-life was 65 hours at 4 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum plasma concentration, along with a long half-life, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) AUC and the maximum plasma concentration (C_{max}) are roughly linearly proportional.

Chronic repeat-dose toxicity and toxicokinetic studies were conducted in the rat (17/26 weeks) and monkey (39 weeks). In the 26-week study of rats, weight loss was observed in the rats given 25 mg/kg/dose of rAvPAL-PEG, SC; corresponding decreased food consumption was observed in the males. The main histological finding was focal-to-multifocal areas of vacuolar degeneration of renal tubule cells in the kidney of 3 males given 25 mg/kg and 3 females given ≥8 mg/kg rAvPAL-PEG. This renal tubule finding persisted in the kidney of both sexes given ≥8 mg/kg/dose at the end of the 12-week recovery phase without evidence of reversibility. Increased vacuolation was observed in histiocytic cells of the liver, spleen, mesenteric lymph node, mandibular lymph node, and adrenal cortex of males given 25 mg/kg/dose and females given ≥8 mg/kg/dose; vacuolation of histiocytic cells in the testes were also observed in males given 25 mg/kg/dose. Both renal tubule vacuolation and histiocytic cell vacuoles

have been associated with PEG-related catabolism and administration of other PEGylated proteins.

In the 39-week, chronic repeat-dose toxicity and toxicokinetic study in monkeys, dose levels of 7.0 and 5.0 mg/kg/dose rAvPAL-PEG were not tolerated in female monkeys and resulted in body weight loss, decreased food consumption, and hypoactivity. The only adverse microscopic finding was arterial inflammation of small and mid-sized arteries in multiple organs and/or tissues in most animals that were given ≥3 mg/kg/dose rAvPAL-PEG SC twice weekly at the end of the dosing phase. The inter-individual organ involvement was highly variable; some animals had findings in one organ and others had multiple organs with arterial inflammation. Additionally, not all arteries had signs of inflammation and arterial inflammation was resolved by the end of the 13-week recovery period. A full analysis of these findings including the involvement of the anti-drug antibodies, antibody isotypes, PEG distribution, and rAvPAL-PEG is ongoing. The no observable adverse effect level (NOAEL) for the 17-week and 39-week studies, in rat and monkey, respectively, is 1 mg/kg/dose. The NOAEL from these studies represents greater than a 100-fold safety factor over the starting dose in PAL-002.

7.2 Previous Clinical Studies

This study is an extension of the second human clinical study with rAvPAL-PEG (Study PAL-002). Study PAL-001, the first-in-human clinical study of rAvPAL–PEG, was designed as a Phase 1, open-label, single-dose, dose-escalation study in **25** subjects, 16 to 50 years old, with PKU. The doses for this study are **based on data from nonclinical studies and PAL-001 and are** 0.001 through 1.0 mg/kg per injection **(not to exceed 2.0 mg/kg/week**, provided no dose-limiting toxicity was observed in **Study PAL-002**.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH (Scriver CR, 2001, McGraw-Hill). Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver CR, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver CR, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated

Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding study (PAL-002). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from PAL-002 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to PEG

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least

80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given **a single dose of** up to 60 mg/kg rAvPAL-PEG **SC**.

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to an Immunologic Reaction

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers, sedimentation rates, and complete blood counts (CBCs).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.) (Harris, 2003, Nat.Rev.Drug Discov.), however, antibodies against PEG may form when PEG is bound to compounds. (Harris, 2003, Nat.Rev.Drug.Discov.) (Richter, 1983, Int.Arch.Allergy Appl.Immunol.) In some instances, development of such antibodies did not result in any significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.). **Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. This may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any**

PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.

7.4.2.1 Systemic Skin Reactions

Two subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. Both of these events were reported as serious and followed administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications.

The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the [redacted]). Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. The subject continues to receive regular doses (every 3 months) of Depo-Provera, and no further reactions have been reported to date (15OCT2009). The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist.

The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. It is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical

properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown **whether administration of PEG-containing drugs, such as** Depo-Provera, has a sensitizing effect to PEG **in rAvPAL-PEG when administered in humans. Because** the exact basis for these reported events **and their duration** is not known, the use of PEG-containing injectable drugs prior to, during, **and after** this study is prohibited as a precautionary measure (refer to Sections 9.3.2 and Section 9.4.8.).

7.4.2.2 Management of Allergic Reactions

Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 2 hours post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection.

In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for antibody testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of allergic symptoms:

- Clearing the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. Acetaminophen or ibuprofen (5-10 mg/kg) may also be administered. An allergy and/or immunology consultation should be sought if necessary.
Information regarding the management of local skin, large local skin, and systemic

reactions is provided in Section 9.1.1. Detailed instructions for the management of allergic reactions are provided in the Study Reference Manual.

7.4.3 Effects of Low Blood Phe

Insufficient Phe intake or excessive rAvPAL-PEG exposure may cause blood Phe concentrations to be too low (eg, < 30 µmol/L). Prolonged low blood Phe concentrations can result in a catabolic state associated with poor growth and altered body functions, including mental and physical alterations, loss of appetite, anemia, rashes, and diarrhea. To ensure safety during this study, subjects will be monitored closely with frequent blood Phe concentration determinations. **If a subject's blood Phe concentration is too low, the subject's diet, current dose of study drug, or both may be adjusted.**

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of **multiple doses of** SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.
- To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of a Phase 2, open-label, dose-finding study (PAL-002) in 35-**50** subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 (0.001 through 1.0 mg/kg per injection), provided no dose-limiting toxicity was observed in PAL-002.

Only subjects who completed participation in PAL-002 will be enrolled into this study. Diet will not be altered during the course of this study, except as necessary for safety **as determined by blood Phe concentrations. If a subject's blood Phe concentration is <120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. For information regarding changes to diet for safety and decreases in dose, refer to Section 9.1.2.2.**

Subjects will be evaluated for safety and for blood Phe concentrations throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.

At least one interim analysis may be performed by the sponsor during the study. In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the **safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor**.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 **subjects'** rAvPAL-PEG dosing will continue in PAL-003 without interruption of dosing. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations **of 120-600** µmol/L. **D**oses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 2.1.1, Schedule of Events. After the subject's blood Phe concentration has been controlled to within a target range (**120-600** µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses **as needed but not to exceed 3x/week (fingerstick tests may be performed more frequently)**.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. **The subject has completed the study through the Month 24 visit.**
4. The study is terminated.

9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG

9.1.1.1 Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product

Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from PAL-002 are excluded from **participation in** this study. **Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in PAL-002 are eligible to participate in this study. S**ubjects may develop **systemic, large local skin, or local skin** reactions after enrollment in PAL-003. **Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.**

9.1.1.2 Definition of Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously

During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows:

Local **skin** reaction:

- **Skin signs or symptoms in 1 affected primary location**, ie, hives, wheals, or swelling or an area of erythema, redness**, induration, pain, or itching** at or near the site of injection.

Large local skin reaction:

- **Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site.**

Systemic reaction **(including generalized skin symptoms)**:

- **Skin and non-skin signs or symptoms** in more than 1 affected **primary** location, ie, cutaneous reaction **in more than 1 area** and/or **anaphylaxis or** any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal**, neurological**.

For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.

[redacted]

[redacted]

9.1.1.3.1 Local Skin Reactions

Subjects who have **had** a local **skin** reaction **(refer to Section 9.1.1.2 for definition) after administration of** rAvPAL-PEG **in PAL-001, PAL-002, or this study** may be premedicated with acetaminophen and antihistamines 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (**Zyrtec [cetirizine]** is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg **Zyrtec** or 25 to 50 mg oral Benadryl **[diphenahydramine]**). Because Benadryl can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver.

Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2).

If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

9.1.1.3.2 Large Local Skin Reactions

Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site. The location of the large local skin reaction relative to the rAvPAL-PEG injection site will determine if a subject may remain in the study and continue to be administered rAvPAL-PEG. An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.

Subjects who have large local skin reactions that are contiguous to the injection site may remain in the study. Subjects who have large local skin reactions that are not contiguous to the injection site may have additional assessments performed (Unscheduled Hypersensitivity Reaction Visit) per Investigator discretion and must then be terminated from the study. A decision to terminate a subject from the study must be agreed to by both the Investigator and the Sponsor's Medical Officer.

9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site

Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may

remain in the study. Large local skin reactions that are contiguous to the injection site should be managed as local skin reactions (refer to Section 9.1.1.3.1). For the remainder of the study, subjects must be premedicated orally with acetaminophen and antihistamines 1 hour prior to study drug dosing. Recommended premedications are acetaminophen and antihistamine (Zyrtec [cetirizine] is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Zyrtec or 25 to 50 mg Benadryl [diphenahydramine]). Because Benadryl can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site

Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction. Subjects who develop large local skin reactions that are not contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may not continue to participate in the study. Subjects may have additional assessments performed (ie, Unscheduled Hypersensitivity Skin Reaction Visit) immediately after the reaction (within 48 hours): serum anti-rAvPAL-PEG antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL immunoglobulin M [IgM], anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG immunoglobulin E [IgE]); serum tryptase level; sedimentation rate; C-reactive protein (CRP), total hemolytic complement (CH50), and complements C_1 and C_4; and clinical laboratory tests (urinalysis, chemistry, hematology). Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4).

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.3 Systemic Reactions

Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG will be terminated from the study. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 48 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity

Skin Reaction Visit): serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE); serum tryptase level (optional; it is recommended that this sample be drawn immediately after reaction); sedimentation rate; CRP, CH50, C_1, and C_4; and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1). Subjects who have a systemic reaction without generalized skin symptoms may have these assessments performed per Investigator discretion. Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4).

9.1.2 Dose Modifications

After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the subject for safety.

9.1.2.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. **Decisions regarding dose increases will depend on blood Phe and drug concentrations**.

Depending upon the response to rAvPAL-PEG in PAL-002, **an individual** subject's dose may be adjusted beginning on Day 1 of PAL-003 as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When **increasing** the dose, a dose may be used that is between the dose levels **(0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, and 1.0 mg/kg)** that were defined in the previous study (PAL-002).
- The dose may be adjusted by increasing the frequency **up to daily**-for **a total weekly dose not to exceed** 2.0 mg/kg**, including subjects who receive more than 1 dose/week**. **Subjects who increase their dose frequency should perform the Interim Dosing Visit assessments (refer to Section 12.3.5 and Table 2.1.2 and Table 2.1.3).**
- When a dose is **increased** (either by **increasing** the dose level or by **increasing** the frequency of dosing), the subject must remain in the clinic for a minimum of 2 hours following the first modified dose administration, and an Investigator will remain on site during this time.
- **Only 1 dose adjustment is allowed every 2 weeks.**

- **Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.**

9.1.2.2 Dose Decrease Methodology

A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if blood Phe concentrations are <120 µmol/L. If a subject's blood Phe level is <120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is <120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (>30% change from baseline) and drug concentrations, as well as toxicity (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for safety (ie, blood Phe concentration <120 µmol/L) or other toxicity that may be improved with a lower, more frequent dose.

A dose should first be reduced by dose level (1.0, 0.6, 0.3, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

9.1.3 Stopping Criteria

If an individual subject exhibits toxicity of **a treatment-emergent** CTCAE grade 3 or greater, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study, restart dosing at the same dose level, or restart dosing at a lower dose level.

In addition, if 2 or more subjects at a dose level exhibit toxicity of **a treatment-emergent** CTCAE grade 3 or greater, further dosing of subjects at that dose level **will be halted** and **no subject will be treated at a** higher dose level until a safety assessment is completed. **In addition, the Food and Drug Administration (FDA) will be notified of this occurrence.**

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in PAL-002.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug.

3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study at any time without prejudice. The Investigator must withdraw from the study any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number **assigned** in PAL-002. **This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.**

Subjects who do not complete the study will not be replaced.

9.4 Treatments

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Dilution instructions are provided in a separate instruction manual.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

At the study site, all IP must be stored at 5 ± 3°C (41 ± 5°F) under the conditions specified in the IB in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 2 mg/kg.

Dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous

measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

The injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.1 as an example. Note this table is for example purposes only. Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. **Study drug will be administered by clinic staff or other qualified and trained study personnel**.

Table 9.4.4.1 Number of Injections Required For an Individual Weighing 80 kg

Dose Group (mg/kg)	Weight (kg)	Volume of Study Drug (mL)[a]	No. of Injections[b]
0.001	80	0.8	1
0.003	80	0.6	1
0.01	80	0.8	1
0.03	80	0.2	1
0.06	**80**	**0.5**	**1**
0.1	80	0.8	1
0.3	80	2.4	2
0.6	**80**	**4.8**	**2 or 3**
1.0	80	8.0	4

[a]Study drug is supplied at a concentration of 10 mg/mL and is then diluted by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume attained following dilution.

[b]Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.

Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency will need to be re-evaluated.

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in PAL-002.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to **120-600** µmol/L will be determined for each subject.

The doses for this study are based upon the results of nonclinical studies and PAL-001. The no observable adverse effect level (NOAELs) from nonclinical studies in rats and

monkeys represents a greater than 100-fold safety factor over the starting dose administered in PAL-002 (0.001 mg/kg).

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered in the morning by clinic staff **or other qualified and trained study personnel**.

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in PAL-002) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior **local skin** reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and antihistamines (refer to Section 9.1.1**). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.**

Subjects who have had a prior **systemic** reaction to rAvPAL-PEG or a PEG-containing product **are excluded from participation in this study** (refer to Section 9.1.1).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study except as necessary for safety. **If a subject's blood Phe concentration is <120 µmol/L, the subject's diet will be adjusted to maintain blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.**

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record

immediately prior to their next dose of study drug. The dietary record will be maintained with the study source documents.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis (Section 2.1) describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.5
Antibody testing	BioMarin[a]	9.7.4.2
PK variables	**BioMarin**	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Urinalysis, pregnancy test (urine), and sedimentation rate	**Local laboratory**	**9.7.5, 9.7.4.1**

Phe, phenylalanine; PK, pharmacokinetic.

[a]**Analysis of the anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.**

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history**, including allergy history,** will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, **weight (kg),** and temperature in degrees Celsius (°C). **Height (cm) will be measured at Screening only.**

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the final follow-up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Month 12 and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn in the morning, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 2.1.1).

In addition, after each administration of study drug, the subject will have a blood Phe measurement by fingerstick **as outlined in Table 2.1.1**. This may be done by the subject at home. **Subjects who have their dose frequency increased will have additional Phe assessments performed as outlined in Table 2.1.2 and Table 2.1.3.**

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (**120-600** µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.

Subjects who have increased their dose frequency to twice weekly or daily will have addition PK assessments performed for 5 weeks after the change in dose regimen (refer to Section 12.3.5 and Table 2.1.2 and Table 2.1.3). BioMarin will perform the analysis.

9.7.4 Safety Variables

9.7.4.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 2.1.1). Female subjects with a positive **serum** pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.4.2 Antibody Testing

Immunogenicity will be assessed by determining antibody response and neutralizing activity with the following measurements: 1) anti-rAvPAL immunoglobulin G (IgG) antibodies, 2) anti-rAvPAL immunoglobulin M (IgM) antibodies, 3) anti-PEG IgG antibodies, 4) anti-PEG IgM antibodies, 5) anti-rAvPAL-PEG immunoglobulin E (IgE) antibodies, and 6) anti-rAvPAL-PEG Enzyme Activity Neutralizing antibodies (NAb). Validated immunogenicity assays will be used per the time points indicated in the Schedule of Events (Table 2.1.1).

BioMarin will perform the analysis **except for anti-rAvPAL-PEG IgE antibodies, which will be assessed by a Contract Research Organization**.

9.7.5 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Specific days for obtaining samples are provided in Table 2.1.1 and in Section 12.
The scheduled clinical laboratory tests are listed in Table 9.7.5.1. A central laboratory will be used for analysis.

Table 9.7.5.1: Clinical Laboratory Tests

Blood Chemistry	**Hematology**	**Urine Tests**[a]	**Other**
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	
BUN	Differential cell count	Protein	
Creatinine	Sedimentation rate[a]	Glucose	
GGT	**CRP**[b]	Bilirubin	Phenylalanine
Total protein		Nitrite	**CH50** [b]
Calcium		Urobilinogen	**C_1** [b]
Sodium		Hemoglobin	**C_4** [b]
Potassium			**Serum tryptase level** [b,c]
Glucose			
Uric acid			
CO_2			
Chloride			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; **CH50, total hemolytic complement**; CO_2, carbon dioxide; **CRP, C-reactive protein;** GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] **To be performed by local laboratory.**

[b] **Perform for Unscheduled Skin Reaction visit only (within 48 hours of reaction).**

[c] **Perform immediately after reaction if possible.**

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

According to the ICH definition, an AE (or adverse experience) is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the IP.”

An adverse drug reaction (ADR) is described by the ICH as “all noxious and unintended responses to a medicinal product related to any dose.” This means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, ie, the relationship cannot be ruled out.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The reporting period for non-serious AEs is the period from the first administration of study drug through the final F/U Visit or at the ETV. If a non-serious AE remains unresolved at the conclusion of the study, the PI and medical monitor will make a joint clinical assessment as to whether continued follow-up of the AE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

The Investigator will assess AEs for severity, for relationship to IP, and as to whether the event meets one or more of the definitions of a serious adverse event (SAE; refer to Section 10.2).

The Investigator will determine the severity of each AE and will record it on the source documents and AE CRF, using the **CTCAE v 3 grades** defined below. **Events that are CTCAE grades 4 and 5 are serious events and require completion of both an SAE form and AE eCRF.**

Grade	Description
1	**Mild**
2	**Moderate**
3	**Severe**
4	**Life threatening or debilitating**
5	**Death related**

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the relationship of an AE to the IP and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	Description
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A SAE is defined as any AE that:

- Results in death.
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization.
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, that is, an AE that occurs in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Is an important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

More than one of the above criteria may apply to any specific event.

The reporting period for SAEs begins earlier than non-serious AEs and is the period from the time of signing of the ICF through 4 weeks after the last dose or at the ETV. SAEs reported to the Investigator outside of this reporting period will be reported to BioMarin if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

Any SAE, whether or not considered related to study drug, will be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate CRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying BioMarin Pharmacovigilance of an SAE. BioMarin may be required to

report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by BioMarin as soon as it becomes available.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: [redacted], **MD**
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history**, including allergy history,** and demographics
- Physical examination
- Vital signs**, including height and weight**
- 12-lead ECG
- Clinical laboratory tests (including hematology, chemistry, and urinalysis)
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs
- Concomitant medications
- Diet query
- **B**lood Phe and plasma tyrosine concentration

For subjects who participated in PAL-002, these assessments may be the same used for the **Week 16** Visit of PAL-002, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done on the third day following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs**, including weight**
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- **Predose PK sample**
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies **(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

12.3.2 Weekly Visits

The following study activities will be performed at the weekly visits beginning with Week 2:

- Vital signs**, including weight**
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- **3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)**
- **Predose PK sample**
    - **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.**
- Predose blood Phe and plasma tyrosine concentration
    - **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.**
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.3 Monthly Visits (Week 4, 8, 12, etc)

Monthly visits consist of all weekly activities and include additional activities.
The following study activities will be performed at the monthly visits beginning with Week 4:

- Vital signs**, including weight**
- Clinical laboratory tests
- **Chest x-ray (Month 12 visit only)**
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- **3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)**
- **Predose PK sample (subjects who are on a stable dosing regimen for ≥3 months only)**
    - **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.**
- Predose blood Phe and plasma tyrosine concentration
    - **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.**
- Serum rAvPAL-PEG antibodies **(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.4 Quarterly Visits (Week 12, 24, 36, etc)

Quarterly visits consist of all **weekly and** monthly activities and include additional activities. The following study activities will be performed at the quarterly visits beginning with Week 12:

- Physical examination
- Vital signs**, including weight**
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- **3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)**
- **Predose PK sample (subjects who are on a stable dosing regimen for ≥3 months only)**
    - **Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.**
- Predose blood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies **(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.5 Interim Dosing Visit

Subjects who increase their dose frequency during the study from 1x/week to 2x/week or daily will have additional assessments performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased. If the Interim Dosing Visit coincides with a weekly, monthly, or quarterly scheduled visit, the scheduled visit assessments should also be performed. The following study activities will be performed for the Interim Dosing Visit:

- **Vital signs, including weight**
- **Injection-site inspection (previous and current injection site)**
- **Assessment of AEs**
- **Concomitant medications**
- **Blood Phe measurement by fingerstick**
    - **This may be done by the subject at home.**
- **PK sample**
    - **Subjects increase their dose frequency to 2x/week will have an additional predose PK sample obtained prior to administration of the first 4 doses (ie, for 2 weeks after the dose frequency increase). For the remaining 3 weeks after the dose frequency increase, a PK sample will be obtained weekly prior to the first dose. Refer to Table 2.1.2 for the Interim Dosing Visit schedule.**
    - **Subjects who have their dose frequency increased to daily will have additional PK samples obtained every day for the first week: 12 hours after the first daily dose and predose every day for the rest of the week. After the first week of daily dosing, subjects will have weekly PK samples obtained for 4 additional weeks. Refer to Table 2.1.3 for the Interim Dosing Visit schedule.**

12.3.6 Unscheduled Hypersensitivity Reaction Visit

Subjects who have a generalized skin reaction after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 48 hours) the reaction. Subjects who have other systemic symptoms or a large local skin reaction that is not contiguous to the injection site (refer to Section 9.1.1) may have these assessments performed per investigator discretion.

- **Injection-site inspection**
- **Clinical laboratory tests**
- **Sedimentation rate**
- **CRP, CH50, C1, and C4**
- **Serum rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**
- **Serum tryptase level**
  - **Perform immediately after reaction if possible.**
  - **Assessment is optional for subjects who have a generalized skin reaction per investigator discretion.**
- **PK sample**
- **Assessment of AEs**
- **Concomitant medications**

Subjects should then return to the clinic 4 weeks after their last dose of rAvPAL-PEG for the Early Termination Visit assessments (refer to Section 12.4).

12.4 Early Termination Visit

The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug.

Subjects who complete an Unscheduled Hypersensitivity Reaction Visit (refer to Section 12.3.6) will be terminated from the study. Subjects will then return 4 weeks after the last dose of study drug for the Early Termination Visit.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs**, including weight**
- 12-lead ECG
- Clinical laboratory tests
- **Chest x-ray**
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- **PK sample**
- **B**lood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies **(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**

12.5 Final Follow-up Visit

The final follow-up (F/U) Visit will occur 1 week after the final dose of study drug.

The following study activities will be performed at the F/U Visit:

- Physical examination
- Vital signs**, including weight**
- 12-lead ECG
- Clinical laboratory tests
- **Chest x-ray**
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- **PK sample**
- **B**lood Phe and plasma tyrosine concentration
- Serum rAvPAL-PEG antibodies **(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)**

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug **in this study and have postdose safety information** will be included in the safety analyses.

Safety will be evaluated on the incidence of AEs, **including serious AEs (SAEs),** and clinically significant changes in vital signs and laboratory test results.

The verbatim terms reported on CRFs to identify AEs will be coded using MedDRA. Treatment-emergent AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. Changes from baseline in vital signs and laboratory test results will be summarized with descriptive statistics.

14.3 Pharmacokinetic Analysis

Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.

Steady-state PK **(predose samples)** of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentrations (**120-600** µmol/L) for a minimum of 2 weeks and no further dose modification is planned.

Should data become available from PAL-002 **that** indicate study drug accumulation should also be measured, additional blood draws **may be added** for PK analysis **as needed but not to exceed 3x/week**.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. **In addition, the proportion of subjects who have maintained target Phe concentrations of 120-600 µmol/L will be summarized by each scheduled time point.**

14.5 Determination of Sample Size

Subjects who participated in PAL-002 may be enrolled into this study. No formal sample size calculation was conducted.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

At least one interim analysis may be performed by the sponsor during the study. **Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).**

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act **independently** in an advisory capacity to BioMarin to monitor **the** safety of rAvPAL-PEG in subjects who participate in the study. Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Study Medical Officer. The Chair will be notified by the Study Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study, on a schedule defined in the DMC Charter, and offer advice on whether or not to proceed, modify or terminate study enrollment on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.3.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the

clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of BioMarin, I confirm that BioMarin, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

M[redacted] Signature 30 Oct 2009 Date

Printed name: [redacted]

BIOMARIN

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical Sciences BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Up to 60 months or until study is terminated
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 3

Date: May 04, 2011

RATIONALE AND SUMMARY OF CHANGES

The primary rationale for amending this protocol is to include a substudy to assess the safety, efficacy, pharmacokinetics (PK), and immune response when rAvPAL-PEG treatment is temporarily discontinued and then re-started in subjects.

rAvPAL-PEG is being assessed as a long-term, chronic treatment for patients with PKU, and there is limited safety, efficacy, PK, and immune response information about the effects of a temporary dose interruption. Therefore, a Substudy has been added to this study to explore the effects of stopping and re-starting rAvPAL-PEG treatment. In this Substudy, up to 10 subjects will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments. Subjects are eligible for participation in the Substudy if they are on a stable rAvPAL-PEG dosing regimen (ie, blood Phe levels have been reached at 60-600 µmol/L) for 4 weeks in this study, have not reported any serious adverse events (SAEs) during this study, and were not enrolled in Study PAL-004.

Currently, there is no information regarding the PK profile of rAvPAL-PEG when blood Phe levels are at 60-600 µmol/L for subjects on a multiple-dose regimen. To address this lack of information, additional PK sampling will be performed prior to the dosing interruption. Upon restarting rAvPAL-PEG, PK sampling will also be performed to allow for collection of drug exposure data (area under the plasma concentration-time curve [AUC] and maximum plasma concentration [C_{max}]), absorption rate, and clearance for subjects who have been previously exposed to rAvPAL-PEG in this Substudy. This information will allow for comparisons to be performed with subjects who were naïve to previous rAvPAL-PEG treatment from the Phase 1 study, PAL-001.

Following participation in the Substudy, subjects will return to the rAvPAL-PEG dose level and frequency that they were receiving prior to the dose interruption and will continue participation in the study until completion.

Because rAvPAL-PEG is a bacterial protein, an immune-mediated reaction and a reduction in efficacy (blood Phe reductions) are possible when rAvPAL-PEG dosing is re-introduced after the dose interruption of the Substudy. However, information from nonclinical studies, antibody results, and safety and efficacy results from the clinical studies to date (JAN2011) have shown that there is no added safety risk or risk of decreased efficacy when rAvPAL-PEG dosing is stopped and then re-started at the same dose level.

- In nonclinical studies using the BTBR Pahenu2 (ENU2) mouse model, an animal model that exhibits characteristics similar to those of PKU patients, a 2-4 week dose-interruption was performed in animals administered 80 mg/kg/week. Following the dose interruption assessment, the animals re-started rAvPAL-PEG dosing at the same dose level (80 mg/kg/week). In this assessment, no safety issues were noted upon re-starting dosing with rAvPAL-PEG and there was an immediate return to stable blood Phe reductions.
- There has not been any immediate immunoglobulin E (IgE)-mediated reactions to rAvPAL-PEG observed following administration of a rAvPAL-PEG injection. All reactions to rAvPAL-PEG have been reported approximately 12-24 hours following administration. To date, 1 out of 33 subjects has tested positive by serology to IgE antibodies without reporting any clinical symptoms to suggest anaphylaxis or a severe systemic reaction. Thus, it is not anticipated that reactions to rAvPAL-PEG are IgE-mediated.
- To date (JAN2011), the clinical experience with re-starting rAvPAL-PEG dosing for subjects from Study PAL-001 who rolled over into Study PAL-002 does not indicate an increased safety risk or a decreased effect on reducing blood Phe levels upon re-introduction of drug.
- Nine subjects who received a single dose of rAvPAL-PEG and completed the Phase 1 study, PAL-001, rolled over into the multiple-dose, Phase 2 study, PAL-002. After a period of several months during which no drug was administered, these 9 subjects re-started rAvPAL-PEG dosing in Study PAL-002 at the same starting doses as was administered in Study PAL-001. There has been no difference to date in the safety profile or efficacy profile of the subjects from Study PAL-001 who rolled over into Study PAL-002 (previously exposed to rAvPAL-PEG) when compared with subjects who were naive to rAvPAL-PEG dosing in Study PAL-002. Although previously exposed subjects had higher and a faster return of anti-PAL and anti-PEG immunoglobulin G (IgG) titers than those of naïve subjects, these results have not been associated with a higher safety risk or efficacy riskbased on information to date.
    - In Study PAL-002, 4 subjects to date have had a protocol-defined (per Protocol Amendment # 3 dated 30APR2010), 2-week washout (no dose

administered) following several weeks of a fixed dose of 0.06 mg/kg/week or 0.1 mg/kg/week rAvPAL-PEG. Following the 2 weeks where no dose was administered, all 4 subjects re-started rAvPAL-PEG dosing at a higher dose with no safety issues reported to date. Blood Phe levels for these 4 subjects have not changed upon re-introduction of dosing.

- Clinical experience with subjects who have had to temporarily halt dosing due to an adverse event also provides information that stopping and re-starting rAvPAL-PEG dosing does not appear to increase the risk to subject safety. To date, 10 subjects participating in either PAL-002 or PAL-003 have had to temporarily stop dosing due to an adverse event. All 10 subjects resumed dosing following resolution of the event or per determination by the Investigator that subject safety would not be jeopardized with re-starting dosing. These 10 subjects resumed dosing at a reduced dose level after approximately 1-7 weeks of no dosing. Upon re-starting dosing, none of these 10 subjects have experienced additional safety issues. Eight subjects continue to receive dosing with rAvPAL-PEG (APR 2011) and have subsequently increased their dose level without additional reported adverse events that have resulted in a subsequent dosing interruption.

In summary, because the risk to subject safety and efficacy is expected to be limited upon re-starting rAvPAL-PEG, dosing will be re-started at the same dose level for subjects in the Substudy. Re-starting rAvPAL-PEG dosing must be performed in the clinic with post-administration observation performed to monitor subject safety. In addition, subjects will continue to be assessed per the safety assessment and stopping criteria defined in the protocol. Dose decreases for safety and increases also will continue to be performed relative to safety as defined in the protocol. In addition, the Substudy objectives have been added to assess the effect of stopping and re-starting rAvPAL-PEG dosing on safety and tolerability, efficacy (blood Phe concentration), PK, and immune response. The PK analysis has also been revised to accommodate for inclusion of the Substudy.

Additional major changes implemented in this protocol amendment are as follows:

- The maximum allowable rAvPAL-PEG dose of 2.0 mg/kg/week has been increased to 5.0 mg/kg/week to allow for further dose increases. This change is based on available safety and blood Phe information to date (JAN2011) from this ongoing study and from the ongoing Phase 2 study, PAL-002. Experience from this study and Study PAL-002to date (JAN2011) indicates that rAvPAL-PEG administered in doses titrated up to a single administration of 2.0 mg/kg/week does not pose a clinically significant risk to subject safety. The most frequently reported AEs in this study and Study PAL-002 have been nonserious injection-site reactions and headache. The reported injection-site reactions have been self-limited and without sequelae. One SAE (dehydration and urticaria) has been reported to date; the event was reported as not related to treatment with rAvPAL-PEG and resolved. Two days after dosing with rAvPAL-PEG, the subject presented with upper respiratory infection symptoms. The subject was started on [redacted] for the upper respiratory infection. Twenty-four hours after starting [redacted] and the third day after dosing with rAvPAL-PEG at 0.1mg/kg, the subject presented with a generalized skin reaction (urticaria). Because the subject had no improvement while on [redacted], [redacted] went to the emergency room and was admitted to the hospital for intravenous fluid resuscitation and the [redacted] was stopped. Since this event, this subject has remained in the study and and has continued dosing with rAvPAL-PEG, escalating the dose to 0.6 mg/kg with no additional events reported to date. Despite rAvPAL-PEG being well tolerated in doses up to 2.0 mg/kg/week, many of the subjects in Study PAL-003 have not yet been able to achieve and sustain blood Phe reductions to clinically significant levels (120-600 µmol/L; JAN2011). While some subjects who have been administered 2.0 mg/kg/week rAvPAL-PEG have had > 50% reduction from baseline in blood Phe levels, they have not yet achieved the protocol-defined target blood Phe of 120-600 µmol/L (JAN2011). It is hypothesized that administration of rAvPAL-PEG doses higher than 2.0 mg/kg/week will reduce the blood Phe levels further to within the protocol-defined range of clinical significance (60-600 µmol/L; target range revised per this protocol amendment) without jeopardizing subject safety. Dose increases up to 5.0 mg/kg/week may be implemented if none of the protocol-defined stopping safety criteria are met. Increases to 5.0 mg/kg/week must be performed in increments, every 2 weeks, per the dose increase instructions and safety assessment and stopping criteria outlined for the study. Upwards titration of dose to 5.0 mg/kg/week will be gradual to mitigate risk to subject safety. Also, subjects who increase dosing to up to 5.0 mg/kg/week will continue to be monitored for safety, blood Phe, PK, and antibodies per the scheduled assessments and procedures.

- The study inclusion criteria have been modified to permit subjects who have completed Study PAL-004 (A Phase 2, Open-Label Study to Evaluate the Safety, Tolerability, and Efficacy of Subcutaneous Dose Levels of rAvPAL-PEG Administered Daily in Subjects with Phenylketonuria) to continue to receive rAvPAL-PEG in this study. Additionally, instruction regarding re-introduction of rAvPAL-PEG dosing has been added for subjects rolling over from Study PAL-004. rAvPAL-PEG treatment will be re-introduced at the same dose level and dosing frequency as was administered at the completion of Study PAL-004. Subjects will be monitored for safety when rAvPAL-PEG dosing is re-started in this study, and subjects will continue to be assessed per the safety assessment and stopping criteria defined in the protocol.
- The target blood Phe level has been revised from 120-600 µmol/L to 60-600 µmol/L to allow for dosing that may provide greater clinical benefit to subjects. Blood Phe levels of approximately 60 ± 13 µmol/L are considered normal per the NIH Consensus Statement, 2000.
- An exploratory objective has been added to assess the relationship of diet and change in blood Phe level in subjects with PKU. Information from a 3-day diet diary will be used to assess dietary Phe intake, as well as all other liquids and food. The study rationale has been revised to include information regarding the relationship of diet in blood Phe concentration following administration of rAvPAL-PEG in subjects with PKU. The study rationale and study outcome measures have also been revised to address this added study objective.
- Information regarding self administration of rAvPAL-PEG has been added. Self-administration of study drug is now allowed by the subject but only if approved by both the Principal Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. This change has been made to increase subject participation and to increase study completion given the long study duration.

Specific revisions to the protocol, including the major changes presented and discussed above, are as follows:

1. Information regarding the PAL-003 Substudy has been added.
    - Substudy objectives have been added for to assess for safety, efficacy, PK, and immune response (refer to the Synopsis [Section 2] and Section 8).
    - The secondary PK objective has been removed; the added PK objective for the Substudy will allow for analysis of PK profile, including clearance, when drug has been stopped and restarted in previously exposed subject who have been administered multiple doses of rAvPAL-PEG (refer to the Synopsis [Section 2] and Section 8), and the PK analysis has been modified to address the Substudy (Section 14.3).

- The Study Rationale has been revised to include information regarding the Substudy (refer to Section 7.3). The nonclinical section (Section 7.1) has also been updated with information regarding nonclinical results relative to stopping and re-starting dosing.
- Information about the Substudy has been added to the Study Design section (refer to the Synopsis [Section 2] and Section 9.1).
- Instruction regarding re-starting rAvPAL-PEG dosing following approximately 4 weeks of no dosing has been added (refer to Section 9.1). rAvPAL-PEG dosing will be re-started at the same dose level and dosing frequency as was administered prior to the dosing interruption. The first dose administered following the dose interruption must be performed in the clinic. A follow-up telephone call will be made to monitor subject safety 24 hours following the return to dosing.
- Information regarding the risks of stopping and re-starting rAvPAL-PEG dosing has been added to the Overall Risks and Benefits section (Section 7.4.3).
- Additional PK sampling has been added immediately prior to the dose interruption, during the dose interruption, and for the first week upon re-starting rAvPAL-PEG. PK sampling timepoints have been added to Section 9.7.3.
- The Study Procedures (Section 12.3.3) and have been modified to include the Substudy PK sampling, and a Substudy Schedule of Events (Table 2.1.2) has been added.

2. The maximum weekly dose amount has been revised from 2.0 mg/kg to 5.0 mg/kg.
   - Information regarding dose increases has been revised; subjects may now increase their dose in increments up to 5.0 mg/kg/week (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, and 4.0 mg/kg/week; refer to the Synopsis [Section 2] and Section 9.1.2.1) provided none of the safety assessment or stopping criteria are met.
   - Information regarding dose decreases has been revised to include decreases in increments for doses starting at 5.0 mg/kg/week (4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, or 0.001 mg/kg; refer to the Synopsis [Section 2] and Section 9.1.2.2).

3. The target blood Phe level has been revised in this amendment from 120-600 µmol/L to 60-600 µmol/L. All subjects in this study may have their rAvPAL-PEG dose increased to achieve a blood Phe reduction to 60-600 µmol/L (refer to the Synopsis [Section 2], Section 9.1, Section 9.1.2, Section 9.4, Section 9.4.6, Section 9.7.3, Section 14.3, and Section 14.4) provided none of the safety assessment or stopping criteria are met.
    - Revisions to the criteria for blood Phe level and dosing modifications have been made to align with the target blood Phe level indicated in Study PAL-002 (60-600 µmol/L) and Study PAL-004 (≤ 60µmol/L). The individualized rAvPAL-PEG dosing will now be based on reductions in blood Phe level to within a range of 60-600 µmol/L. Previously, the individualized rAvPAL-PEG dosing was based on reductions in blood Phe levels to ≥ 30% of baseline levels.
4. Subjects who have completed Study PAL-004 are now eligible for enrollment into Study PAL-003.
    - The inclusion criterion #1 and exclusion criterion #8 have been revised to allow for inclusion of subjects who have completed Study PAL-004 (refer to the Synopsis [Section 2], Section 9.3.1, and Section 9.3.2).
    - Instruction regarding re-starting rAvPAL-PEG dosing has been added for subjects who completed Study PAL-004 and who are re-starting rAvPAL-PEG dosing in this study (refer to the Synopsis [Section 2]and Section 9.1) following approximately 4 weeks of no dosing. rAvPAL-PEG dosing will be re-started at the same dose level and frequency as was administered upon completion of PAL-004. A follow-up telephone call will be made to monitor subject safety 24 hours following the return to dosing.
    - The number of study sites and the number of participating subjects have been increased to accommodate for inclusion of subjects who completed Study PAL-004 (refer to Synopsis [Section 2] and Section 9.1).
5. An exploratory objective has been added to assess the long-term effects of diet and change in blood Phe concentration following administration of rAvPAL-PEG (refer to Section 8).
    - Information regarding analysis of diet and change in blood Phe concentration has been added to the Study Rationale (Section 7.3), the Exploratory Efficacy Variable (Section 9.7.4), and the Statistical Analysis (Section 14.4) sections.

6. Information regarding diet has been revised. Subjects should no longer modify diet for blood Phe concentrations ≤ 120 µmol/L. Diet should now remain stable during the study unless directed otherwise by the Investigator in consultation with the Sponsor's Medical Officer (refer to Synopsis [Section 2], Section 9.1, and Section 9.6).
7. Information regarding the allowed number of missed doses has been added to allow subjects who miss dosing due to reasons other than non-compliance to continue to participate in this long-term extension study. Subjects who miss more than 2 consecutive weekly doses (subjects who are dosed weekly) or 3 consecutive daily doses (subjects who are dosed daily) are allowed to continue in the study (refer to Section 9.4.10). Subjects who miss doses due to subject decision, Sponsor decision, or Investigator decision may continue to participate in this study but must continue to perform the scheduled study assessments for blood Phe levels, pharmacokinetics (PK), antibodies, and safety.
    - Instructions have been added regarding how to re-start rAvPAL-PEG dosing following a dosing interruption that is not due to subject non-compliance. rAvPAL-PEG dosing will be re-started at the same dose level and frequency as was administered prior to the dosing interruption.
    - Subjects should continue to perform the scheduled study assessments during any dosing interruption.
8. Information regarding the safety assessment criteria (referred to as the stopping criteria in the previous protocol amendment, A2) has been revised to align with the criteria in the other Phase 2 studies, PAL-002 and PAL-004.
    - The definition of the safety assessment criteria has been revised to better monitor subject safety. Dosing will be halted for subjects who have a Common Terminology Criteria for Adverse Events (CTCAE) grade ≥ 3 event, including a systemic reaction that is assessed by the Investigator as related to treatment with rAvPAL-PEG (Synopsis [Section 2] and Section 9.1.3).
    - The definition of the stopping criteria has been revised to better monitor subject safety. Dosing will be halted for the subjects at the same dose level until a safety evaluation has been completed if 2 or more subjects at a dose level have a Common Terminology Criteria for Adverse Events (CTCAE) grade ≥ 3 event, including a systemic reaction that is assessed by the Investigator as related to treatment with rAvPAL-PEG (Synopsis [Section 2] and Section 9.1.4).

- Subjects who are discontinued from study treatment due to safety will now be asked to remain in the study and to continue to perform the remaining scheduled assessments to continue to collect information, including safety information, until study completion (refer to the Synopsis [Section 2] and Section 9.3.3).

9. Based on clinical experience to date (JAN2011) from this ongoing study and ongoing Study PAL-002, reductions in blood Phe to normal levels (ie, 60± 13 µmol/L) have not had clinical consequences to subject safety. Therefore, information regarding the possible toxic effects of low blood Phe has been removed.
    - The Summary of Overall Risks and Benefits (Section 7.4) has been revised; information regarding the possible toxic effects of low blood Phe (Section 7.4.3) has been removed.
10. The protocol is being amended to allow for self-administration of rAvPAL-PEG by subjects. This change is to facilitate subject recruitment and subject convenience in this long-term study. Subjects may self administer study drug at home only if the subject meets the protocol-specified requirements, per approval of both the Principal Investigator and the Sponsor's Medical Officer, and if adequate training is demonstrated. Additional information will be provided in the PAL-003 Subject Self-Administration Training Materials (refer to the Synopsis [Section 2] and Section 9.4).
11. Information regarding systemic reactions and large local reactions has been revised based on clinical experience from the ongoing Phase 2 study, PAL-002 (refer to Section 9.1.1) and to align with information in the protocols for the other Phase 2 studies, PAL-002 and PAL-004.
    - Subjects who have had a systemic reaction or a large local reaction not contiguous to the injection site during the study are no longer required to discontinue from study treatment. Following the reaction, subjects will temporarily halt treatment and be required to complete the assessments of the Unscheduled Hypersensitivity Reaction visit, including assessment of immunoglobulin E (IgE). Subjects who do not have an IgE-mediated reaction may resume treatment with study drug per the discretion of the Investigator and the Sponsor's Medical Officer with premedication and close monitoring. Subjects who have an IgE-mediated reaction should discontinue from further study treatment but continue to complete the remaining study assessments. Information for including these subjects in this study has been provided in Section 7.4.2.

- An additional assessment to monitor subject safety (ie, a drug-specific immunoglobulin E [IgE] assay) has been developed and validated for use during the study. Subjects who had a systemic reaction in Study PAL-002 or Study PAL-004 must be premedicated with antihistamine and acetaminophen prior to each administration of rAvPAL-PEG in this study (Section 9.1.1.3.3).
- The exclusion criterion (#4) has been revised to allow participation of subjects who have had a previous systemic reaction per the discretion of the Principal Investigator and the Sponsor's Medical Officer (refer to the Synopsis [Section 2] and Section 9.3.2).
- An optional skin biopsy of the affected and not affected area has been added to the Unscheduled Hypersensitivity Reaction Visit for subjects who have had a systemic reaction or a large local reaction not contiguous to the injection site. The biopsy is optional per subject consent and should be taken within 1 week of the reaction (refer to Section 9.1.1.3.3, Section 12.3.7, and Table 2.1.1). The biopsy will allow for further monitoring of subject safety.

12. To monitor for subject safety, assessments have been added for subjects who have rAvPAL-PEG dosing administered outside of a scheduled clinic visit, including subjects who are on a daily dosing regimen. AEs, concomitant medications, and an inspection of the injection site will be assessed (refer to Section 12 and Table 2.1.1).
13. The safety assessments performed during the Unscheduled Hypersensitivity Reaction visit are now mandatory for subjects who have a systemic reaction or a large local reaction that is not contiguous with the injection site. This revision has been made to align with information in the protocols for the other Phase 2 studies, PAL-002 and PAL-004. Subjects who have been deemed appropriate to continue study treatment must perform the Unscheduled Hypersensitivity Reaction visit assessments prior to resuming dosing with rAvPAL-PEG (refer to Section 9.1.1, Section 12, and Table 2.1.1).
14. The secondary objective regarding PK has been removed; rAvPAL-PEG drug exposure will be assessed per the objectives added for the Substudy [Section 2] and Section 8). In addition, the PK assessments for subjects who have reached target Phe levels for at least 2 weeks have been clarified; sampling will be performed once per week for 3 consecutive weeks (refer to Section 9.7.3).
15. The Safety Reporting section has been revised for clarity and to align with information in the protocols for the other Phase 2 studies, PAL-002 and PAL-004(refer to Section 10).

16. The required visits that must be performed in the clinic have been clarified; weekly visits are not required to be performed in the clinic if performed by a home healthcare professional. This change will allow for increased subject compliance (refer to Section 12 and Table 2.1.1).
17. The duration of the study has been extended from 24 months to up to 60 months to allow for extended collection of long-term safety information from subjects who are administered rAvPAL-PEG (refer to the Synopsis [Section 2] and Section 9.1).
18. The PK assessments have been removed from the Interim Dosing Visit for subjects who increase their dose frequency (refer to Section 12and Table 2.1.3and Table 2.1.4) because PK information will now be obtained from the Substudy.
19. Information regarding the timing of dose administration and sampling for PK and blood Phe has been revised; dose administration and sampling no longer need to be performed in the morning but must be performed prior to dose administration. This change has been made to allow for feasibility of dosing for both sites and subjects (refer to Section 9.4.6.1, Section 9.7.2.1, and Section 9.4.6.1).
20. Results from the completed Phase 1 study, PAL-001, have been added (Section 7.2.1). Information regarding Phase 2 Study PAL-002 has been updated and information regarding Phase 2 Study PAL-004 has been added to the Background section (refer to Section 7.2).
21. Because the systemic allergic skin reactions reported in ongoing Study PAL-002 and this ongoing study to date (JAN2011) have been assessed as variants of delayed allergic reactions and do not typically occur until at least 12-24 hours after dose administration, the immediate post-dose observation period has been reduced. The post-administration observation period has been revised from 2 hours to 30-60 minutes (refer to the Synopsis [Section 2], Section 7.4.2.2, Section 7.4.3, Section 9.1, Section 9.1.1.3.3, Figure 9.1.1.3.1, Section 9.1.2.1, and Section 9.4.10). Subjects who have a systemic reaction regardless of when it occurs relative to dosing are still required to report to the clinic as soon as possible for assessment of safety and to have additional laboratory tests performed (ie, the Unscheduled Hypersensitivity Reaction Visit; refer to Section 12.3.7).
    - Subjects who increase their dosing frequency are no longer required to have the post-administration observation period if the increased frequency does not result in an increase in the total weekly dose amount administered (refer to the Synopsis [Section 2] and Section 9.1.2.1).

22. The maximum amount of dose allowed per injection of 1.0 mg/kg has been removed to align with information in the protocols for the other Phase 2 studies, PAL-002 and PAL-004; the dose amount and dosing frequency will be managed per the dose increases and dose decreases outlined per the revised information in this amendment (refer to the Synopsis [Section 2], Section 9.1, and Section 9.1.2).
23. Phe (fingerstick) assessments have been added for subjects who modify their dose due to safety. These additional assessments will allow for blood Phe concentration to be assessed more frequently (daily for 3 days) following a change in dose (refer to Synopsis [Section 2], Section 9.1.2.1, and Section 12).
24. A chest X-ray has been added to the Screening visit (refer to Section 12 and Table 2.1.1) to monitor for subject safety.
25. Nonclinical information has been updated with results from the chronic, repeat-dose studies and updates made to the Investigators Brochure (version 4, 1MAR2011; refer to Section 7.1).
26. The Schedule of Events (Table 2.1.1) and the Study Procedure section (Section 12) have been updated to reflect the changes to this amendment.
27. Administrative revisions have been made to improve clarity and consistency.

Specific revisions to the text of each section of the protocol (since the protocol amendment finalized on 30OCT2009), including the Synopsis, are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding studies (Study PAL-002 and Study PAL-004). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To assess the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU.
- To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU.
- To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU.
- To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

STUDY DESIGN AND PLAN:

This is a long-term extension of the Phase 2, open-label, dose-finding studies, PAL-002 and PAL-004, in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity is observed in PAL-002 or PAL-004.

Only subjects who completed the protocol-defined study drug regimen in PAL-002 or PAL-004 will be enrolled into this study. Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing in this study. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study.Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigaor in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 and PAL-004 subjects' rAvPAL-PEG dosing will continue in PAL-003. In

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The subject has completed the study through the Month 60 visit.
4. The study is terminated.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

PAL-003 Substudy

A subset of up to 10 subjects who are already enrolled and who have consented to participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.

Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

For subjects from PAL-002 only, an individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg)) or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject.
- The dose may be adjusted by increasing the frequency up to dailyfor a total weekly dose not to exceed 5.0 mg/kg.Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit).
- When a dose is increased, the subject must be observed for 30-60 minutes following the

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.

- Only 1 dose (level or frequency) adjustment is allowed every 2 weeks.
- Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.

Dose Decrease Methodology

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for safety (ie, any adverse event that may be improved with a lower, more frequent dose).

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

Safety Assessment Criteria:

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

Stopping Criteria:

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will be evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

NUMBER OF SUBJECTS PLANNED:

Approximately 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation and all protocol-defined study drug in PAL-002 or PAL-004.
2. Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

14 days prior to the administration of study drug.

3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.

4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction of generalized rash may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002 or PAL-004.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1. 5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1. 5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Upon enrollment into this study, subjects from PAL-002 will be dosed with the same or higher dose than the dose that was administered upon completion of PAL-002. Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 6~~12~~0-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.

DURATION OF TREATMENT:

Up to 60 months.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:
None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, and anti-PEG-PAL IgE) will be assessed.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in PAL-002 or PAL-004 may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseline, of vital signs, ECG results, and X-ray results will also be provided.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

For subjects who have received any study drug in this study with any post-treatment blood Phe concentration measurements and diet diary information, the relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will be explored.

Substudy Analysis:

For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

2.1 Schedule of Events

Table 2.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]				Final F/U Visit	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours; then perform Early Term. procedures
		Week 1	Weekly	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)			
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	1 week after final dose	4 weeks after final dose	
Informed consent	X							
Medical history, including allergy history, and demographics	X							
Physical examination[e]	X	X			X	X	X	
Vital signs and weight[e]	X	X	X			X	X	
12-lead ECG	X					X	X	
Clinical laboratory tests[f]	X			X		X	X	X[g]
Sedimentation rate		X			X	X	X	X
Chest x-ray	X			X (12-month visit only)		X	X	
Urine pregnancy test[h]	X	X		X		X	X	
Injection-site inspection[i, k]		X (postdose)	X			X	X	X
Adverse events[j, k]	X←----------					----------→X	X	X
Concomitant medications[k]	X	X	X			X	X	X
Diet query	X	X	X			X	X	
3-Day diet diary[l]			X			X	X	
Serum antibodies		X		X		X	X	X
Plasma Phe and plasma tyrosine[m]	X	X	X			X	X	
Plasma Phe (fingerstick)[n]			(3 days postdose)					
Plasma PK sample[o]		X	X			X	X	X
Administer IP[p]		X	X					

Assessments and Events	Screening[a]	Treatment Period[b,c]				Final F/U Visit	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours; then perform Early Term. procedures
		Week 1	Weekly	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)			
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	1 week after final dose	4 weeks after final dose	
Skin biopsy (optional; affected and not affected area)								X
Serum tryptase level [q]								X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] It is preferable to perform PAL-003 Screening assessments the same day as PAL-002 Week 16 visit or PAL-004 Week 16 visit so there is no interruption of study drug. If performed on the same day, PAL-002 Week 16 visit or PAL-004 Week 16 visit data may be used. If not, PAL-003 Day 1 must occur ≤ 28 days after PAL-003 Screening.

[b] Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. Monthly visits must be performed in the clinic. Dose modifications must be performed in the clinic. Additional visits may occur if deemed necessary to monitor AEs or blood Phe, adjust dosing etc.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Unscheduled hypersensitivity reaction assessments are mandatory for subjects who have a generalized skin reaction. Subjects who have other systemic symptoms or who have a large local skin reaction that is not contiguous to the injection site may have these assessments performed per investigator discretion. Assessments should be performed within 48 hours of the reaction Subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer with close monitoring. Subjects who remain in the study and continue study treatment must be premedicated. Refer to Section 9.1.1.3.3and Section 12.3.7.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include hematology, chemistry, and urinalysis. Refer to Table 9.7.6.1.

[g] Subjects who have a systemic reaction or a large local reaction not contiguous with the injection site should be assessed for CRP, CH50, C_1, C_3, and C_4 within 48 hours of the reaction.

[h] If positive or equivocal, perform serum pregnancy test.

[i] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm).

[j] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[k] Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.

[m] Samples should be drawn at least 2.5 hours after a meal. If dose is modified by increasing dose level, additional blood Phe concentration testing should be done on the third day following dose adjustment.

[n] May be done by the subject at home. Samples should be taken at least 2.5 hours after a meal. Will be performed daily for 3 consecutive days after each dose increase.

[o] The PK blood samples will be collected after the subject has achieved and maintained target blood Phe concentrations (60-600 µmol/L) for a minimum of 2 weeks. Once this target is achieved, once weekly predose PK samples will be collected for 3 consecutive weeks.

[p] Dosing is up to 5.0 mg/kg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency will need to be re-evaluated.

[q] Mandatory for subjects who have a systemic reaction or a large local skin reaction that is not contiguous to the injection site. Take sample immediately after reaction if possible.

Table 2.1.2: Schedule of Events-Substudy

Substudy Assessments and Events	Substudy Period[a]				
	Final Dose (Day 1)	Day 8	Day 15	Day 22	Re-start Dose (Day 29)
Physical examination[b]	X	X	X	X	X
Vital signs and weight	X	X	X	X	X
Clinical laboratory tests	X	X	X	X	X
Sedimentation rate	X	X	X	X	X
Urine pregnancy test[c]	X	X	X	X	X
Injection-site inspection[d]	X (Pre & postdose)				X (postdose)
Adverse events	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Diet query	X	X	X	X	X
3-Day diet diary[e]	X	X	X	X	X
Serum antibodies	X	X	X	X	X
Plasma Phe and plasma tyrosine	X	X (8, 12, and 16 hours/Day 1, 24 hours/Day 2, 48 hours/Day 3, 72 hours/Day 4, 96 hours/Day 5, 144 hours/Day 7, 168 hours/Day 8, 216 hours/Day 10 after Final Dose)			X (Predose and 24 hours/Day 2, 48 hours/Day 3, 72 hours/Day 4, 120 hours/Day 6, 168 hours/Day 8 after Re-start Dose
Plasma PK sample	X (Predose)	X (8, 12, and 16 hours/Day 1, 24 hours/Day 2, 48 hours/Day 3, 72 hours/Day 4, 96 hours/Day 5, 144 hours/Day 7, 168 hours/Day 8, 216 hours/Day 10 after Final Dose)			X (Predose and 24 hours/Day 2, 48 hours/Day 3, 72 hours/Day 4, 120 hours/Day 6, 168 hours/Day 8 after Re-start Dose
Administer IP	X				X

AE, adverse event; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine.

[a] On days that a dose is given, all procedures should be performed predose, except where noted.

[b] Complete physical examinations to include the evaluation of all major body systems.

[c] If positive or equivocal, perform serum pregnancy test.

[d] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm).

[e]It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug on Substudy Days 1 and 29. On Substudy Days 8, 15, and 22, it is preferable that the subject complete the diary 3 days immediately prior to the next study visit.

Table 2.1.3: Interim Dosing Visit Schedule, Subjects Whose Dose is Increased to 2x/Week

Assessments and Events	Subjects Who Are Administered rAvPAL-PEG 2x/Week Treatment Period[a, b]									
	First Week After Dose Adjustment		Second Week After Dose Adjustment		Third Week After Dose Adjustment		Fourth Week After Dose Adjustment		Fifth Week After Dose Adjustment	
	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose	First Dose	Second Dose
Vital signs and weight	X	X	X	X	X	X	X	X	X	X
Injection-site inspection	X	X	X	X	X	X	X	X	X	X
Adverse events[c]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Blood Phe (fingerstick)[d]	X	X	X	X	X	X	X	X	X	X

AE, adverse event; D, day; PK, pharmacokinetics; Phe, phenylalanine.

[a] The additional Interim Dosing Visit assessments should be performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased to 2x/week. All assessments should be performed predose unless specified otherwise.

[b] If the Interim Dosing Visit coincides with a regularly scheduled visit, the scheduled visit assessments should also be performed.

[c] If any injection site reaction is observed, the corresponding AE data must specify injection location (identify current vs. previous visit's injection, e.g,. right arm vs. left arm).

[d] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

Table 2.1.4: Interim Dosing Visit Schedule, Subjects Whose Dose is Increased to Daily

	Subjects Who Are Administered rAvPAL-PEG Daily Treatment Period[a, b]					
	First Day After Dose Adjustment	First Week After Dose Adjustment	Second Week After Dose Adjustment	Third Week After Dose Adjustment	Fourth Week After Dose Adjustment	Fifth Week After Dose Adjustment
Assessments and Events	Dose 1	Doses 2-7	Doses 8-14	Doses 15-21	Doses 22-29	Doses 30-36
Vital signs and weight	X	X	X	X	X	X
Injection-site inspection	X	X	X	X	X	X
Adverse events[c]	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X
Blood Phe (fingerstick)[d]	X	X	X	X	X	X

AE, adverse event; D, day; PK, pharmacokinetics; Phe, phenylalanine.

[a] The additional Interim Dosing Visit assessments should be performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased to daily. All assessments should be performed predose unless specified otherwise.

[b] the Interim Dosing Visit coincides with a regularly scheduled visit, the scheduled visit assessments should also be performed.

[c] If any injection site reaction is observed, the corresponding AE data must specify injection location (identify current vs. previous visit's injection, e.g,. right arm vs. left arm).

[d] May be done by the subject at home. Samples should be taken in the morning, at least 2.5 hours after a meal.

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

[redacted]

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram

GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
NOAEL	no observable adverse effect level
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan

SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), urinalysis, and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively,

and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given $\geq$ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU patients who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002 and Study PAL-004) based on data from the completed clinical study, PAL-001, and clinical experience to date (JAN2011) from this ongoing study and the other ongoing Phase 2 study, PAL-002.

7.2.1 Phase 1 Study PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.

- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002 and PAL-004

Currently, rAvPAL-PEG is being investigated in two Phase 2 clinical trials (PAL-002 and PAL-004). To date (JAN2011), a total of 33 subjects have been administered rAvPAL-PEG in Study PAL-002 and initiation and recruitment of clinical sites is underway for Study PAL-004.

In PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU), approximately 55 subjects will be enrolled and will be administered multiple doses of rAvPAL-PEG up to 5.0 mg/kg/week. PAL-002 is a 2-part study. In Part 1, subjects will be assigned to 1 of 4 rAvPAL-PEG dose cohorts (0.001, 0.003, 0.01, 0.1 mg/kg, or 1.0 mg/kg). rAvPAL-PEG will be administered at a fixed weekly dose of 0.001 mg/kg (Cohort 1), 0.003 mg/kg (Cohort 2), 0.01 mg/kg (Cohort 3), 1.0 mg/kg (Cohort 4), or 0.1 mg/kg (Part 1 Substudy) for 8 weeks. In Part 2, all subjects who complete Part 1 will be administered an adjustable dose of rAvPAL-PEG for up to an additional 8 weeks. The dose administered in Part 2 may be adjusted by dose level and/or dose frequency to reach a target blood Phe concentration of 60-600 µmol/L. Study PAL-003 (Long-Term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU) is an extension study of PAL-002 and PAL-004. Once subjects have completed Study PAL-002 or PAL-004, they have the option to enroll into PAL-003 and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months.

Study PAL-002 was initiated in September 2009 and is ongoing. The primary objective of this study is to evaluate the effect of multiple doses of rAvPAL-PEG on blood Phe concentrations in subjects with PKU for up to 16 weeks of treatment. The secondary objectives of the study are (1) to evaluate the safety and tolerability of SC injections of multiple dose levels of rAvPAL-PEG, (2) to evaluate the antibody response to rAvPAL-PEG, and (3) to evaluate the PK profile of rAvPAL-PEG in subjects with PKU. The study protocol was amended (30APR2010) to include a substudy of an additional rAvPAL-PEG dose level

(0.1 mg/kg/week) that reduced blood Phe concentrations to clinically significant levels in the Phase 1 study, PAL-001. The protocol was amended again (28MAR2011) to increase the starting dose for Cohort 4 from 0.03 mg/kg/week to 1.0 mg/kg/week. To date (JAN2011), 33 subjects have been enrolled into the study and all 10 subjects of the substudy have been dosed with 0.1 mg/kg rAvPAL-PEG. Additional information about the safety reported to date (JAN2011) is provided in Section 7.4.2.

Study PAL-004 is designed to determine if daily administration of rAvPAL-PEG at daily dose levels (0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day) is safe and effective in reducing and maintaining consistent blood Phe concentrations in subjects with PKU. It is hypothesized that a higher starting dose and frequency will reduce blood Phe concentrations to near-normal levels in a timely manner and will provide the greatest clinical benefit. Considering the concentration and volume of drug that is administered subcutaneously, the only way to increase the rAvPAL-PEG dose is via daily administration. Therefore, in Study PAL-004, a higher starting dose of 0.4 mg/kg will be administered 5 days a week (referred to as daily), a dosing regimen that has been observed to be tolerated and to reduce blood Phe concentrations to clinically meaningful levels in previously exposed subjects in this study. Following completion of Study PAL-004, subjects have the option to enroll into this study and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months.

In this study, subjects in PAL-002 and PAL-004 will continue to be administered the same rAvPAL-PEG that was administered in PAL-002 or PAL-004. If the safety, PK, and blood Phe concentration results from either PAL-002 or PAL-004 indicate that the appropriate level of PEGylation and dosing regimen differs from that administered in PAL-002 or PAL-004, the PEGylation and/or dosing regimen administered in this study may change.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003,

Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. To date (JAN2011), there is limited information on the effects of a rAvPAL-PEG dose interruption in subjects with PKU. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments. There is no information regarding the pharmacokinetic (PK) profile of rAvPAL-PEG when blood Phe levels are at 60-600 µmol/L for subjects on a multiple-dose regimen. To address this lack of information, additional PK sampling will be performed prior to the dosing interruption. Upon restarting rAvPAL-PEG, PK sampling will also be performed to allow for collection of drug exposure data (area under the plasma concentration-time curve [AUC] and maximum plasma concentration [C_{max}]), absorption rate, and clearance for subjects who have been previously exposed to rAvPAL-PEG in this Substudy. This information will allow for comparisons to be performed with

subjects who were naïve to previous rAvPAL-PEG treatment from the Phase 1 study, PAL-001.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary.

This study is an extension of the dose-finding studies (PAL-002 and PAL-004). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from PAL-002 or PAL-004 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to PEG

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to an Immunologic Reaction

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers, sedimentation rates, and complete blood counts (CBCs).

In Study PAL-001, there were 11 subjects (out of 25 subjects in the study) with skin-related reactions (injection-site bruising, erythema, pain, rash, swelling, and urticaria) that occurred following the single administration of rAvPAL-PEG. For all of these subjects, the reactions were generalized skin reactions or injection-site skin reactions that did not compromise other organs and were not life-threatening. These suspected antibody-mediated responses were nonserious and mild or moderate in severity. These subjects are not considered to be at significant risk for antibody-mediated reactions with injection of rAvPAL-PEG during PAL-002, PAL-004, or this study; however, specific precautions for these subjects will be taken during this study to monitor subject safety (refer to Section 9.1.1).

In Study PAL-002, 33 subjects have been dosed to date (JAN2011), including at dose levels of 2.0 mg/kg/week (or 0.4 mg/kg/5 days a week). Most reactions reported in these subjects have been nonserious and mild or moderate in severity. There has been 1 SAE reported to date; the event was reported as not related to treatment with study drug. There have been no reports of anaphylaxis to date (JAN2011). Subjects who have a systemic reaction during this study will undergo a series of assessments to monitor safety, including assessment of IgE antibodies, prior to determining if dosing may resume. Additionally, subjects who have systemic reactions will be premedicated and will be monitored closely for safety (refer to Section 9.1.1.3.3 for additional information regarding continued treatment of subjects with systemic reactions during the study).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.), however, antibodies against PEG may form when PEG is bound to compounds. (Harris, 2003, Nat.Rev.Drug Discov.),(Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in any significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.) Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. This may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.

7.4.2.1 Systemic Skin Reactions

Two out of 25 subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. One of these events was reported as serious, and the other was reported as nonserious; both events were reported following administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications.

The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the [redacted]. Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. Upon study completion, the subject continued to receive regular doses (every 3 months) of Depo-Provera, and no further reactions were reported. The second

subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist. See Section 7.2.1 for the results from Study PAL-001.

The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. The results from nonclinical studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys indicated the formation of anti-rAvPAL antibody titers that did not correspond with observations of injection-site reactions. Quantitation of anti-PEG titers were not assessed in the chronic repeat-dose studies in the rat and monkey. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to, during, and after this study is prohibited as a precautionary measure (refer to Section 9.3.2 and Section 9.4.8.).

7.4.2.2 Management of Allergic Reactions

Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30-60 minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection. Subjects who qualify for self administration of study drug will be provided with emergency resuscitation instructions (refer to Section 9.4 and the Subject Self-Administration Training Materials).

In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for antibody testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of allergic symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. Acetaminophen or ibuprofen (5-10 mg/kg) may also be administered. An allergy and/or immunology consultation should be sought if necessary. Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.1.1. Detailed instructions for the management of allergic reactions are provided in the Study Reference Manual.

7.4.3 Effects of Stopping and Re-Starting rAvPAL-PEG Treatment

The effects of starting, stopping, and re-starting rAvPAL-PEG dosing on safety and blood Phe reductions previously achieved through rAvPAL-PEG dosing are not well defined, and there is limited clinical experience with rAvPAL-PEG. Allergic reactions are a common and expected clinical outcome of re-starting biologic drugs, such as rAvPAL-PEG, and may occur when subjects re-start rAvPAL-PEG dosing. However, no information reported from the ongoing clinical studies, including this study, or nonclinical results point to an increased safety risk or decreased efficacywhen rAvPAL-PEG dosing is stopped and then re-started at the same dose level.

- Nonclinical experience in the BTBR *Pah*^{enu2} (ENU2) mouse model indicates that dosing interruptions of 2-4 weeks for mice administered rAvPAL-PEG 80 mg/kg/week had no increased safety risk upon re-starting dosing and there was an immediate return to stable blood Phe reductions (refer to Section 7.1).

- Antibody results from the ongoing Phase 2 study, PAL-002, and this ongoing study to date (JAN2011) have not indicated an immune response that points to an increased safety risk or decreased efficacy upon re-introduction of drug. There has not been any immediate immunoglobulin E (IgE)-mediated reactions to rAvPAL-PEG observed following administration of a rAvPAL-PEG injection. All reactions to rAvPAL-PEG have been reported approximately 12-24 hours following administration. To date, 1 out of 33 subjects has tested positive by serology to IgE antibodies without reporting any clinical symptoms to suggest anaphylaxis or a severe systemic reaction. Thus, it is not anticipated that reactions to rAvPAL-PEG are IgE-mediated.
- To date (JAN2011), the clinical experience with re-starting rAvPAL-PEG dosing for subjects from Study PAL-001 who rolled over into Study PAL-002 does not indicate an increased safety risk or a decreased effect on reducing blood Phe levels upon re-introduction of drug.
    - Nine subjects who received a single dose of rAvPAL-PEG and completed the Phase 1 study, PAL-001, rolled over into the multiple-dose, Phase 2 study, PAL-002. After a period of several months during which no drug was administered, these 9 subjects re-started rAvPAL-PEG dosing in Study PAL-002 at the same starting doses as was administered in Study PAL-001. There has been no difference to date in the safety profile or efficacy profile of the subjects from Study PAL-001 who rolled over into Study PAL-002 (previously exposed to rAvPAL-PEG) when compared with subjects who were naive to rAvPAL-PEG dosing in Study PAL-002. Although previously exposed subjects had higher and a faster return of anti-PAL and anti-PEG IgG titers than those of naïve subjects, these results have not been associated with a higher safety risk or efficacy riskbased on information to date.
    - In Study PAL-002, 4 subjects to date have had a protocol-defined (per Protocol Amendment # 3 dated 30APR2010), 2-week washout (no dose administered) following several weeks of a fixed dose of 0.06 mg/kg/week or 0.1 mg/kg/week rAvPAL-PEG. Following the 2 weeks where no dose was administered, all 4 subjects re-started rAvPAL-PEG dosing at a higher dose with no safety issues reported to date. Blood Phe levels for these 4 subjects have not changed upon re-introduction of dosing.
    - Clinical experience with subjects who have had to temporarily halt dosing due to an adverse event also provides information that stopping and re-starting rAvPAL-PEG dosing does not appear to increase the risk to subject safety. To date, 10 subjects participating in either PAL-002 or PAL-003 have had to

temporarily stop dosing due to an adverse event. All 10 subjects resumed dosing following resolution of the event or per determination by the Investigator that subject safety would not be jeopardized with re-starting dosing. These 10 subjects resumed dosing at a reduced dose level after approximately 1-7 weeks of no dosing. Upon re-starting dosing, none of these 10 subjects have experienced additional safety issues. Eight subjects continue to receive dosing with rAvPAL-PEG (APR 2011) and have subsequently increased their dose level without additional reported adverse events that have resulted in a subsequent dosing interruption.

In this study, measures will be taken to reduce the risk to subject safety upon re-starting rAvPAL-PEG dosing while still allowing for reductions in blood Phe level to clinical significance (blood Phe reduction to 60-600 µmol/L). To mitigate the possible safety risks upon re-starting rAvPAL-PEG dosing, re-introduction of dosing should be performed in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call should be made to subjects 24 hours following re-starting of dosing to monitor subject safety. Subjects will also continue to be assessed per the safety assessment and stopping criteria defined in Section 9.1.3 and Section 9.1.4, and dose decreases will continue to be performed relative to safety as defined in Section 9.1.2.2. To mitigate the possible reduced effect on blood Phe level upon re-starting rAvPAL-PEG, subjects in this study who interrupt dosing whether per the protocol (ie, subjects participating in the Substudy; refer to Section 9.1) or for other reasons (refer to Section 9.4.10) will re-start rAvPAL-PEG dosing at the same dose level.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To assess the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU.
- To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU.
- To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU.
- To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of the Phase 2, open-label, dose-finding studies, PAL-002 and PAL-004, in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity was observed in PAL-002 or PAL-004.

Only subjects who completed the protocol-defined study drug regimen in PAL-002 or PAL-004 will be enrolled into this study. Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing in this study. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigator in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to each clinic visit for review with the clinical study staff.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self administration. Additional information is provided in the Subject Self-Administration Training Materials.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 and PAL-004 subjects' rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 μmol/L. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 2.1.1, Table 2.1.2, Table 2.1.3 and Table 2.1.4 (Schedule of Events). After the subject's blood Phe concentration has been controlled to within a target range (60-600 μmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

A subject will continue in PAL-003 until one of the following occurs:

1. The subject withdraws consent and discontinues from the study.
2. The subject is discontinued from the study at the discretion of the Investigator.
3. The subject has completed the study through the Month 60 visit.
4. The study is terminated.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

A subset of up to 10 subjects who are already enrolled and who have consented to participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose

interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption. To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.

9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG

Subjects who qualify for self administration of study drug will be provided with information and instruction with regard to management of local and systemic reactions (refer to the Subject Self-Administration Training Materials).

9.1.1.1 Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product

Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from PAL-002 or PAL-004 are excluded from participation in this study. Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in PAL-002 or PAL-004 are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or antihistamines 1 hour prior to study drug dosing for the remainder of the study. The premedication dosage will be standard. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable). Subjects may develop systemic, large local skin, or local skin reactions after enrollment in PAL-003. Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.

9.1.1.2 Definition of Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously

During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows:

Local skin reaction:

- Skin signs or symptoms in 1 affected primary location, ie, hives, wheals, or swelling or an area of erythema, redness, induration, pain, or itching at or near the site of injection.
- Large local skin reaction:
- Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site.
- Systemic reaction (including generalized skin symptoms):
- Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological; and/or a fever attributed to treatment with rAvPAL-PEG (≥ 100.4°F or ≥ 38°C).

For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.

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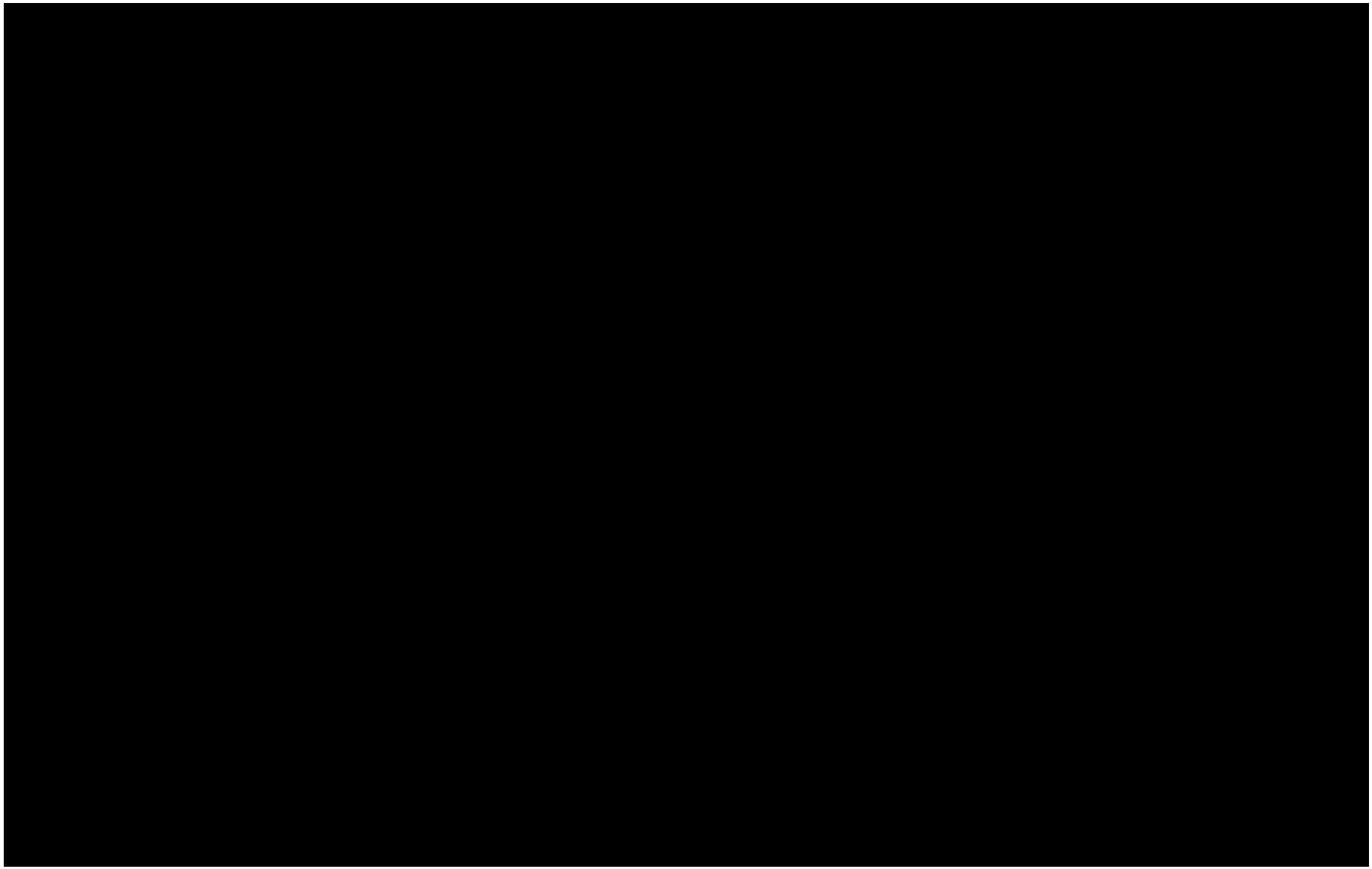

9.1.1.3.1 Local Skin Reactions

Subjects who have had a local skin reaction (refer to Section 9.1.1.2 for definition) after administration of rAvPAL-PEG in this study may continue if the symptoms do not worsen and no other symptoms have developed but may be premedicated with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug dosing. Because antihistamines can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver.

Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2).

If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from study treatment.

9.1.1.3.2 Large Local Skin Reactions

Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects may be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing.

9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site

Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. For the remainder of the study, subjects may be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site

Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction (refer to Section 9.1.1.3.3). An example of

a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.3 Systemic Reactions

Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG must stop further administrations of rAvPAL-PEG and must immediately return to the clinic for safety assessments . Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 48 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Reaction Visit): serum antibodies (anti--PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti rAvPAL-PEG IgE, anti-rAvPAL-PEG neutralizing antibodies); serum tryptase level (it is recommended that this sample be drawn immediately after reaction); sedimentation rate; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1).

Following completion of the Unscheduled Systemic Reaction Visit, administration of rAvPAL-PEG may resume up to 1 week following the onset of the reaction if the systemic reaction is not IgE-mediated and the subject's safety will not be further compromised with resumed dosing. rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects must be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section 9.1.2. Subjects must be

premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

9.1.2 Dose Modifications

9.1.2.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

For subjects from PAL-002, an individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg) or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg.Subjects who increase their dose frequency should perform the Interim Dosing Visit assessments (refer to Section 12.3.6 and Table 2.1.3 and Table 2.1.4).
- When a dose is increased, the subject must be observed for 30-60 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose (level or frequency) adjustment is allowed every 2 weeks.
- Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.

9.1.2.2 Dose Decrease Methodology

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for safety (ie, any adverse event that may be improved with a lower, more frequent dose).

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

9.1.3 Safety Assessment Criteria

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

9.1.4 Stopping Criteria

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation and all protocol-defined study drug in PAL-002 or PAL-004.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.

4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction of generalized rash may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination fro PAL-002 or PAL-004.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study treatment should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study treatment include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in PAL-002 or PAL-004. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from PAL-002 will be dosed with the same or higher dose that was administered upon completion of PAL-002. Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed to by the PI and the Study Medical Officer, based upon the subject's response to doses.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects may be eligible to self administer study drug if he or she meets the following criteria:

- The subject is on a stable dosing regimen for 2 weeks.
- The subject has not experienced any CTCAE Grade 3 or higher adverse event.
- The subject has not experienced any hypersensitivity reaction to rAvPAL-PEG for at least 4 weeks.
- The subject has no cognitive impairments that may increase the safety risk of self administration per the assessment of the PI.
- The subject has been approved for self administration of study drug by the Sponsor's Medical Officer.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with epinephrine and has been trained on when and how to administer it.

Qualified study site personnel will train each eligible subject on all procedures for self administration of study drug under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self administer a dose at home. The patient will see a study site nurse

or home healthcare nurse in person every week to ensure that the subject continues to perform all self-administration procedures correctly, to assess of adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 2.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials.

Subjects who are eligible for self administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.
- How to safely use and dispose of syringes used for injections of study drug.
- How to use a new syringe and vial every time drug is administered.
- How to care for their injection site after an injection of study drug.
- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine.
- Who to contact at the study site in case of an emergency.

The PI or the Sponsor's Medical Officer may request that self administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplyingthe study drug to subjects who qualify for self administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Dilution instructions are provided in a separate instruction manual.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

IP must be stored at 5 ±3°C (41 ± 5°F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg.

Dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

The injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.1 as an example. Note this table is for example purposes only. Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff or other qualified and trained study personnel.

Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1. Information for dosing of subjects who enroll into this study after completing Study PAL-004 is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

Table 9.4.4.1: Number of Injections Required For an Individual Weighing 80 kg

Dose Group (mg/kg)	Weight (kg)	Volume of Study Drug (mL)[a]	No. of Injections[b]
0.001	80	0.8	1
0.003	80	0.6	1
0.01	80	0.8	1
0.03	80	0.2	1
0.06	80	0.5	1
0.1	80	0.8	1
0.3	80	2.4	2
0.6	80	4.8	2 or 3
1.0	80	8.0	4

aStudy drug is supplied at a concentration of 10 mg/mL and is then diluted by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume.

[b]Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.

Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may need to be re-evaluated (refer to Section 9.4.10).

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in PAL-002 or PAL-004.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study and the ongoing study, PAL-002, to date (JAN2011).

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in PAL-002 or PAL-004) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and/or antihistamines (refer to Section 9.1.1). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior systemic reaction to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.1.1).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1. Information for dosing of subjects who enroll into this study after completing Study PAL-002 or Study PAL-004 is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator in consultation with the Sponsor's Medical Officer. Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 2.1.1, Table 2.1.2, Table 2.1.3 and Table 2.1.4) during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study treatment per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigaor in consultation with the Sponsor's Medical Officer.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next dose of study drug. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis (Section 2.1) describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a]	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Urinalysis, pregnancy test (urine), and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, weight (kg), and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Month 12, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 2.1.1, Table 2.1.2, Table 2.1.3 and Table 2.1.4).

In addition, after each administration of study drug, the subject will have a blood Phe measurement by fingerstick as outlined in Table 2.1.1, Table 2.1.2, Table 2.1.3 and Table 2.1.4. This may be done by the subject at home.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG. For subjects participating in the Substudy, PK sampling will be performed as follows:

- When dosing is stopped, PK sampling will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16 hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours.
- When dosing is re-started, PK sampling will be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours.

BioMarin will perform the anlaysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits. Subjects will be instructed to record all

food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each week. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 2.1.1 and Table 2.1.2). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgE, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1.3.3). Validated immunogenicity assays will be used per the time points indicated in the Schedule of Events (Table 2.1.1 and Table 2.1.2).

BioMarin will perform the analysis except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not any abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Specific days for obtaining samples are provided in Table 2.1.1, Table 2.1.2, and in Section 12. The scheduled clinical laboratory tests are listed in Table 9.7.6.1. A central laboratory will be used for analysis.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Bilirubin	Phenylalanine
Total protein		Nitrite	CH50 [b]
Calcium		Urobilinogen	C_1 [b]
Sodium		Hemoglobin	C_4 [b]
Potassium			C_3 [b]
Glucose			Serum tryptase level [b,c]
Uric acid			Sedimentation rate[a]
CO_2			CRP[b]
Chloride			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by local laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory.

[b] Perform for Unscheduled Hypersensitivity Reaction visit only (within 48 hours of reaction).

[c] Perform immediately after reaction if possible.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a patient administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study period during which all non-serious AEs and SAEs will be reported begins after informed consent is obtained and the first adminstration of study drug and continues until 4 weeks following the last administration of study drug or the last visit of the treatment period (refer to Section 12). After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. Resolution of AEs (with dates) should be documented on the appropriate CRF page(s) and in the subject’s medical record.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on patient/event outcome or action criteria usually associated

with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)
4	Life threatening or debilitating: consequences; urgent intervention indicated[b]
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	Description
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
- Note: Life-threatening refers to an event that places the patient at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a patient exposed to IP prior to conception or during pregnancy.
- Is an important medical event or reaction.

The reporting period for SAEs begins after informed consent is obtained, and continues until 4 weeks following the last administration of study drug or End of Treatment Visit.

All SAEs, whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form to BioMarin Pharmacovigilance (BPV).). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available.The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the patient is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the patient's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (ie, modified dose amount or frequency not defined in protocol).
- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.5 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to each clinic visit for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including hematology, chemistry, and urinalysis)
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in PAL-002 or PAL-004, these assessments may be the same as those used for the Week 16 Visit of PAL-002 or the Week 16 visit ofPAL-004, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs, including weight
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)
    - For subjects enrolling into this study from Study PAL-004, first dose of rAvPAl-pEG must be given in the clinic setting and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.

12.3.2 Weekly Visits

Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. However, dose modifications must be peformed in the clinic. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

The following study activities will be performed at the weekly visits beginning with Week 2:

- Vital signs**,** including weight
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)
- PK sample
- For subjects participating in the Substudy, refer to Section 12.3.3.
- Blood Phe and plasma tyrosine concentration
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)
    - For subjects participating in the Substudy, refer to Section 12.3.3.

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.3 Substudy Visits (Weekly)

The following study activities will be performed on Day 1 of the Substudy (ie, final dose prior to interruption), Day 8, Day 15, Day 22, and Day 29 (ie, restart administration of study drug) for subjects participating in the Substudy:

- Physical examination
- Vital signs, including weight
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (predose; postdose on Day 1; during weekly visit on Day 8, 15, 22; and postdose on Day 29)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies,)
- Blood Phe and plasma tyrosine concentration
    - When dosing is stopped, sampling for blood Phe assessment only will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16 hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours.
    - When dosing is re-started, sampling for blood Phe assessment only will be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours.
- PK sample
- For subjects participating in the Substudy, additional PK sampling will be performed upon the final dose prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG. Refer to Section 9.7.3
- Administer study drug (Day 29 only)

- For subjects participating in the Substudy, administration of rAvPAL-PEG will not be performed for approximately 4 consecutive weeks regardless of their dosing regimen (dose level and frequency). After approximately 4 weeks of no dosing, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of re-administration must be given in the clinic setting, and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.

12.3.4 Monthly Visits (Week 4, 8, 12, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Monthly visits consist of all weekly activities and include additional activities and must be performed in the clinic. The following study activities will be performed at the monthly visits beginning with Week 4:

- Vital signs, including weight
- Clinical laboratory tests
- Chest x-ray (Month 12 visit only)
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)
- PK sample
- For subjects participating in the Substudy, refer to Section 12.3.3.
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)

- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)
    - For subjects participating in the Substudy, refer to Section 12.3.3.

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.5 Quarterly Visits (Week 12, 24, 36, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Quarterly visits consist of all weekly and monthly activities and include additional activities. The following study activities will be performed at the quarterly visits beginning with Week 12:

- Physical examination
- Vital signs, including weight
- Clinical laboratory tests
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)
- PK sample
    - For subjects participating in the Substudy, refer to Section 12.3.3.
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)

- For subjects participating in the Substudy, refer to Section 12.3.3.

In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.

12.3.6 Interim Dosing Visit

Subjects who increase their dose frequency during the study from 1x/week to 2x/week or daily will have additional assessments performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased. If the Interim Dosing Visit coincides with a weekly, monthly, or quarterly scheduled visit, the scheduled visit assessments should also be performed. The following study activities will be performed for the Interim Dosing Visit:

- Vital signs, including weight
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Blood Phe measurement by fingerstick
  - This may be done by the subject at home.

12.3.7 Unscheduled Hypersensitivity Reaction Visit

Subjects who have a systemic reaction, including a generalized skin reaction, or a large local skin reaction that is not contiguous to the injection site after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 48 hours) the reaction.

- Injection-site inspection
- Clinical laboratory tests
- Sedimentation rate
- CRP, CH50, C1, C3, and C4
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies)
- Serum tryptase level
- Perform immediately after reaction if possible.
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction)

- PK sample
- Assessment of AEs
- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction Visit assessments, the subject may resume treatment with study drug per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. Subjects who have had an IgE-mediated reaction must be discontinued from further study drug administration. Subjects who do not have an IgE-mediated reaction may resume study drug administration under close monitoring; subjects must be premedicated orally with acetaminophen and/or antihistamines 1 hour prior to study drug dosing for the remainder of the study (refer to Section 9.1.1.3.3).

12.4 Early Termination Visit

The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who terminate from study treatment early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs, including weight
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications

- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

12.5 Final Follow-up Visit

The final follow-up (F/U) Visit will occur 1 week after the final dose of study drug.

The following study activities will be performed at the F/U Visit:

- Physical examination
- Vital signs, including weight
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Final Follow-up Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseine, of vital signs, ECG results, and X-ray results will also be provided.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).Should data become available from PAL-002 or PAL-004 that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the Sponsor’s Medical Officer.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis. Information regarding this exploratory analysis will be provided in the Statistical Analyais Plan.

For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored. Information regarding the analysis will be provided in the Statistical Analyais Plan.

14.5 Determination of Sample Size

Subjects who participated in PAL-002 or PAL-004 may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these

circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study. Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Study Medical Officer. The Chair will be notified by the Study Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study, on a schedule defined in the DMC Charter, and offer advice on whether or not to proceed, modify or terminate study enrollment on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.3.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the

clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of BioMarin, I confirm that BioMarin, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

[redacted] May 4, 2011

Medical Monitor Date

Printed name: [redacted]

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale. Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by strikethrough font.

Section No./Title		
Section 2/Synopsis (Study Sites)	Approximately 20~~8~~ centers in the United States	4
Section 2/Synopsis (Study Rationale)	The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. Phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the dose-finding studies~~y~~ (Study PAL-002 and Study PAL-004). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects. Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments. Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore,	1, 5

Section No./Title		
	an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary.	
Section 2/Synopsis (Objectives)	The secondary objectives of the study are: • To evaluate the immune ~~antibody~~ response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU. • ~~To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.~~ The exploratory objective of the study is as follows: • To assess the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU. The Substudy objectives are as follows: • To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU. • To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU. • To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU. • To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.	1, 5, 14
Section 2/Synopsis (Study Design and Plan)	This is a long-term extension of ~~a~~thePhase 2, open-label, dose-finding studies,~~y (Study~~ PAL-002 and PAL-004, in ~~35-50~~ approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5~~1~~.0 mg/kg/week~~per injection~~), provided no dose-limiting toxicity i~~was~~s observed in	1-4, 6, 27

Section No./Title		
	PAL-002 or PAL-004. Only subjects who completed the protocol-defined study drug regimen ~~participation~~ in PAL-002 or PAL-004 will be enrolled into this study. Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing in this study. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Diet ~~will~~ should not be altered during the course of this study ~~, except as necessary for safety as determined by blood Phe concentrations. If a subject's blood Phe concentration is < 120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.~~ Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigaor in consultation with the Sponsor's Medical Officer.To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff. Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration. Subjects will be evaluated for safety ~~and for blood Phe concentrations~~ throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4~~3~~. ~~At least one interim analysis may be performed by the sponsor during the study.~~ In addition	

Section No./Title		
	to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 and PAL-004 subjects' rAvPAL-PEG dosing will continue in PAL-003 ~~without interruption of dosing~~. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 6~~12~~0-600 µmol/L. Doses will be evaluated on an individual basis. Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (6~~12~~0-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer~~but not to exceed 3x/week (fingerstick tests may be performed more frequently)~~. A subject will continue in PAL-003 until one of the following occurs: 1. The subject withdraws consent and discontinues from the study. 2. The subject is discontinued from the study at the discretion of the Investigator. 3. The subject has completed the study through the Month ~~24~~60 visit. 4. The study is terminated. Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety. PAL-003 Substudy A subset of up to 10 subjects who are already enrolled and who have consented to	

Section No./Title		
	participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption. To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.	
Section 2/Synopsis (Dose Modifications)	Dose Modifications: ~~After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the subject for safety.~~ Dose Increase Methodology: Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations. ~~Blood Phe levels will be measured 3 days after each dose increase (fingerstick is~~	2, 3, 21, 22

Section No./Title		
	~~acceptable); more frequent or daily blood Phe measurements may be performed.~~ For subjects from PAL-002 only~~Depending upon the response to rAvPAL PEG in PAL-002,~~ an individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows. • The dose may be increased by increments of no more than 10-fold higher than the subject's current dose. • When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, ~~and~~ 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg)~~ that were defined in the previous study (PAL-002).~~or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) and drug concentrations, as well as any adverse events (any hypersensitivity reaction) for an individual subject. • The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5~~2~~.0 mg/kg~~, including subjects who receive more than 1 dose/week.~~ Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit). • When a dose is increased ~~(either by increasing the dose level or by increasing the frequency of dosing)~~, the subject must ~~remain in the clinic~~be observed for ~~a minimum of~~ 30-60 minutes~~2 hours~~ following the first modified dose administration~~, and an Investigator will remain on site during this time~~.Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount. • Only 1 dose (level or frequency) adjustment is allowed every 2 weeks. • Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements	

Section No./Title		
	may be performed. Dose Decrease Methodology ~~A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if a subject's blood Phe concentrations < 120 µmol/L. If a subject's blood Phe level is < 120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is < 120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.~~ All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L~~> 30% change from baseline~~) a~~nd drug concentrations~~, as well as any adverse events~~toxicity~~ (any hypersensitivity reaction). Dose decreases may occur for safety (ie, ~~blood Phe concentration < 120 µmol/L) or other toxicity~~any adverse event that may be improved with a lower, more frequent dose). A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.	
Section 2/Synopsis (~~Stopping~~ Safety Assessment Criteria)	**Safety Assessment**~~Stopping~~ Criteria: If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same	8

Section No./Title		
	dose level, or restart dosing at a lower dose level. **Stopping Criteria:** ~~In addition, if~~ 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will be evaluated and ~~halted and no subjects will be treated at a higher dose level until~~ a safety assessment will be ~~is~~ completed. In addition, the Food and Drug Administration (FDA) ~~will~~ may be notified of this occurrence if appropriate.	
Section 2/Synopsis (Number of Subjects Planned)	~~35 50~~Approximately 100 subjects.	4
Section 2/Synopsis (Diagnosis and All Criteria for Inclusion and Exclusion)	Individuals eligible to participate in this study must meet all of the following criteria: 1. Must have completed participation and all protocol-defined study drug in PAL-002 or PAL-004. Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments. 2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug. 3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation. 4. A prior reaction that included systemic symptoms (eg, generalized hives,	4, 11

Section No./Title		
	respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction of generalized rash may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study. 8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002 or PAL-004.	
Section 2/Synopsis (Investigational Product, Dose, Route, and Regimen)	rAvPAL-PEG ~~doses~~ will be ~~administered SC~~ provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product. Upon enrollment into this study, subjects from PAL-002 will be dosed with the same or higher dose than the dose that was administered upon completion of PAL-002. Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. ~~t~~The dosage that provides control of blood Phe concentrations within the target range of 6~~12~~0-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses.	1, 3, 4, 27
Section 2/Synopsis (Duration of Treatment)	Up to 60 months.~~Extension of multiple dosing will continue until one of the following occurs:~~	17

Section No./Title		
	~~1. The subject withdraws consent and discontinues from the study.~~ ~~2. The subject is discontinued from the study at the discretion of the Investigator.~~ ~~3. The subject has completed the study through the Month 24 visit.~~ ~~4. The study is terminated.~~	
Section 2/Synopsis (Criteria for Evaluation)	Immunogenicity: The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies) will be assessed. Safety: Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and~~or~~ laboratory test results. Pharmacokinetic: Plasma concentrations of rAvPAL-PEG will be measured ~~when steady-state levels of Phe are attained~~for subjects participating in the Substudy.	22, 27
Section 2/Synopsis (Statistical Methods)	**Sample Size:** Subjects who participated in PAL-002 or PAL-004 may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy. **Safety Analysis:** All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those	1, 3, 4, 5, 27

Section No./Title		
	subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug.~~Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.~~Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseline, of vital signs, ECG results, and X-ray results will also be provided. **Efficacy Analysis:** Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis. Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of ~~120~~60-600 µmol/L will be summarized by each scheduled time point. **Exploratory Analysis:** For subjects who have received any study drug in this study with any post-treatment blood Phe concentration measurements and diet diary information, the relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will be explored. **Substudy Analysis:** For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored.	

Section No./Title		
	Pharmacokinetic Analysis: ~~Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.~~ ~~Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (120-600 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Should data become available from PAL-002 that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed r~~but not to exceed 3x/week.~~ Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearane (CL/F).	
Tables 2.1/Schedule of Events	Table 2.1.1, Schedule of Events, has been revised to reflect change made to the study. Table 2.1.2, Schedule of Events-Substudy, has been added. Tables 2.1.~~2~~3 and 2.1.~~3~~4, Interim Dosing Visit Schdules, have been revised to remove the PK sampling.	13, 16, 18, 19, 22, 23, 24, 26
Section 4/List of Abbreviations and Definitions of Terms	C_3 complement 3 C_4 complement 4 rAvPAL-PEG ~~rAv~~ recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG	27
Section 6/Investigators and Administrative Structure	Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol (rAvPAL and rAvPAL-PEG) IgE antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a	27

Section No./Title		
	central laboratory, with the exception of erythrocyte sedimentation rate (ESR), urinalysis, and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations. Pharmacokinetic (PK) analysis will be performed by BioMarin.	
Section 7.1/Nonclinical Studies	The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 µM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 µM to < 200 µM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 µM over the entire week, which continued for a total of 15 weeks. In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis et al., Long-term correction of PKU in the	1, 25

Section No./Title		
	Pahenu2 mouse by mutant and chemically modified forms of Phenylalanine Ammonium Lyase. The American Society of Human Genetics, 57th Annual Meeting, 2007). Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously. Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional. Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a	

Section No./Title		
	26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given ≥ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey. ~~The murine model of phenylketonuria (PKU) BTBR*Pah*^{enu2} (ENU2), a mouse line that is deficient in phenylalanine hydroxylase (PAH) activity (Shedlovsky, 1993, Genetics), was used for in vivo screening and pharmacodynamic studies. This mouse model exhibits characteristics similar to those seen in PKU patients, including hyperphenylalaninemia (baseline plasma phenylalanine [Phe] concentrations of 1000 to 2000 µM) and hypopigmentation. Weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG (approximately 4 IU/mouse) for greater than 2 to 3 months lowered and stabilized plasma Phe concentration from approximately 2000 µM to less than 200 µM. A similar profile of Phe reduction was also seen in ENU2 mice administered wild-type AvPAL-PEG (4 IU/mouse) over 8 weeks. In all these studies, regardless of the molecule, an attenuated Phe-lowering response was usually seen between Weeks 2 and 7. However, plasma Phe concentrations became stable at concentrations below 200 µM from Week 7 onward. In addition to the reduction of plasma Phe, a dose-related darkening in coat color occurred,~~	

Section No./Title		
	~~indicating the biosynthesis of melanin, which was previously inhibited when plasma Phe could not be metabolized and plasma Phe concentrations were high. When administration of AvPAL-PEG was stopped for 2 to 7 weeks and then reinitiated with weekly administrations, no attenuated response was observed and Phe concentrations remained low and stable until the next administration.~~ ~~The safety of rAvPAL-PEG was evaluated in safety pharmacology studies (respiratory, central nervous system [CNS] and cardiovascular [CV]) and toxicity with toxicokinetic studies (single and 28-day repeated dose) in rats and cynomolgus monkeys. Overall, in the nonclinical studies, no anaphylactoid-like reactions or injection-site reactions were noted. No specific polyethylene glycol (PEG)-related histological findings were observed during the 28-day repeated dose studies.~~ ~~Findings in cynomolgus monkeys dosed with a single administration of 60 mg/kg rAvPAL-PEG included reduced food consumption, dehydration, body weight loss, hypoactivity, hypothermia,decreased plasma Phe concentration, decreased protein synthesis, and gastrointestinal (GI) lesions. Plasma Phe concentrations were also reduced to below the level of detection in the 4 and 12 mg/kg dose groups, but without similar toxicological consequences, implying that the physiological regulation of Phe levels may be an important factor for influencing morbidity.~~ ~~The main finding in the 28-day study was the possible drug-related observation of minimal to slight degeneration of blood vessels of predominantly medium-sized muscular arteries with a non-dose-dependent involvement of different organs observed in 1 male in the 0.1 mg/kg and 1 male and 1 female in the 1.0 mg/kg rAvPAL-PEG dose groups. No degeneration of the arteries was observed in either the control or animals administered 0.01 mg/kg rAvPAL-PEG.~~ ~~The half-life of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and~~	

Section No./Title		
	~~250 mg/kg, was 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC) dose linearity. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the half-life was 65 hours at 4 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum plasma concentration, along with a long half-life, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) AUC and the maximum plasma concentration (C_{max}) are roughly linearly proportional.~~ ~~Chronic repeat-dose toxicity and toxicokinetic studies were conducted in the rat (17/26 weeks) and monkey (39 weeks). In the 26-week study of rats, weight loss was observed in the rats given 25 mg/kg/dose of rAvPAL-PEG, SC; corresponding decreased food consumption was observed in the males. The main histological finding was focal-to-multifocal areas of vacuolar degeneration of renal tubule cells in the kidney of 3 males given 25 mg/kg and 3 females given ≥ 8 mg/kg rAvPAL-PEG. This renal tubule finding persisted in the kidney of both sexes given ≥ 8 mg/kg/dose at the end of the 12-week recovery phase without evidence of reversibility. Increased vacuolation was observed in histiocytic cells of the liver, spleen, mesenteric lymph node, mandibular lymph node, and adrenal cortex of males given 25 mg/kg/dose and females given ≥ 8 mg/kg/dose; vacuolation of histiocytic cells in the testes were also observed in males given 25 mg/kg/dose. Both renal tubule vacuolation and histiocytic cell vacuoles have been associated with PEG-related catabolism and administration of other PEGylated proteins.~~ ~~In the 39-week, chronic repeat-dose toxicity and toxicokinetic study in monkeys, dose~~	

Section No./Title		
	~~levels of 7.0 and 5.0 mg/kg/dose rAvPAL-PEG were not tolerated in female monkeys and resulted in body weight loss, decreased food consumption, and hypoactivity. The only adverse microscopic finding was arterial inflammation of small and mid-sized arteries in multiple organs and/or tissues in most animals that were given ≥ 3 mg/kg/dose rAvPAL-PEG SC twice weekly at the end of the dosing phase. The inter-individual organ involvement was highly variable; some animals had findings in one organ and others had multiple organs with arterial inflammation. Additionally, not all arteries had signs of inflammation and arterial inflammation was resolved by the end of the 13-week recovery period. A full analysis of these findings including the involvement of the anti-drug antibodies, antibody isotypes, PEG distribution, and rAvPAL-PEG is ongoing. The no observable adverse effect level (NOAEL) for the 17-week and 39-week studies, in rat and monkey, respectively, is 1 mg/kg/dose. The NOAEL from these studies represents greater than a 100-fold safety factor over the starting dose in PAL-002.~~	
Section 7.2/Previous Clinical Studies	~~This study is an extension of the second human clinical study with rAvPAL-PEG (Study PAL-002). Study PAL-001, the first-in-human clinical study of rAvPAL-PEG, was designed as a Phase 1, open-label, single-dose, dose-escalation study in 25 subjects, 16 to 50 years old, with PKU. The doses for this study are based on data from nonclinical studies and PAL-001 and are 0.001 through 1.0 mg/kg per injection (not to exceed 2.0 mg/kg/week, provided no dose-limiting toxicity was observed in Study PAL-002.~~ The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU patients who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002 and Study PAL-004) based on data from the completed clinical study,	20

Section No./Title		
	PAL-001, and clinical experience to date (JAN2011) from this ongoing study and the other ongoing Phase 2 study, PAL-002, and are 0.001 through 5.0 mg/kg/week.	
Section 7.2.1/Phase 1 Study: PAL-001	The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations. The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG. Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows: • rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg. • For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L. • Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003,	20

Section No./Title		
	0.01, and 0.03 mg/kg. • For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.	
Section 7.2.2/Phase 2 Studies: PAL-002 and PAL-004	Currently, rAvPAL-PEG is being investigated in two Phase 2 clinical trials (PAL-002 and PAL-004). To date (JAN2011), a total of 33 subjects have been administered rAvPAL-PEG in Study PAL-002 and initiation and recruitment of clinical sites is underway for Study PAL-004. In PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU), approximately 55 subjects will be enrolled and will be administered multiple doses of rAvPAL-PEG up to 5.0 mg/kg/week. PAL-002 is a 2-part study. In Part 1, subjects will be assigned to 1 of 4 rAvPAL-PEG dose cohorts (0.001, 0.003, 0.01, 0.1 mg/kg, or 1.0 mg/kg). rAvPAL-PEG will be administered at a fixed weekly dose of 0.001 mg/kg (Cohort 1), 0.003 mg/kg (Cohort 2), 0.01 mg/kg (Cohort 3), 1.0 mg/kg (Cohort 4), or 0.1 mg/kg (Part 1 Substudy) for 8 weeks. In Part 2, all subjects who complete Part 1 will be administered an adjustable dose of rAvPAL-PEG for up to an additional 8 weeks. The dose administered in Part 2 may be adjusted by dose level and/or dose frequency to reach a target blood Phe concentration of 60-600 µmol/L. Study PAL-003 (Long-Term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU) is an extension study of PAL-002 and PAL-004. Once subjects have completed Study PAL-002 or PAL-004, they have the option to enroll into PAL-003 and to continue to receive doses of rAvPAL-PEG	20

Section No./Title		
	for up to an additional 60 months. Study PAL-002 was initiated in September 2009 and is ongoing. The primary objective of this study is to evaluate the effect of multiple doses of rAvPAL-PEG on blood Phe concentrations in subjects with PKU for up to 16 weeks of treatment. The secondary objectives of the study are (1) to evaluate the safety and tolerability of SC injections of multiple dose levels of rAvPAL-PEG, (2) to evaluate the antibody response to rAvPAL-PEG, and (3) to evaluate the PK profile of rAvPAL-PEG in subjects with PKU. The study protocol was amended (30APR2010) to include a substudy of an additional rAvPAL-PEG dose level (0.1 mg/kg/week) that reduced blood Phe concentrations to clinically significant levels in the Phase 1 study, PAL-001. The protocol was amended again (28MAR2011) to increase the starting dose for Cohort 4 from 0.03 mg/kg/week to 1.0 mg/kg/week. To date (JAN2011), 33 subjects have been enrolled into the study and all 10 subjects of the substudy have been dosed with 0.1 mg/kg rAvPAL-PEG. Additional information about the safety reported to date (JAN2011) is provided in Section 7.4.2. Study PAL-004 is designed to determine if daily administration of rAvPAL-PEG at daily dose levels (0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day) is safe and effective in reducing and maintaining consistent blood Phe concentrations in subjects with PKU. It is hypothesized that a higher starting dose and frequency will reduce blood Phe concentrations to near-normal levels in a timely manner and will provide the greatest clinical benefit. Considering the concentration and volume of drug that is administered subcutaneously, the only way to increase the rAvPAL-PEG dose is via daily administration. Therefore, in Study PAL-004, a higher starting dose of 0.4 mg/kg will be administered 5 days a week (referred to as daily), a dosing regimen that has been observed to be tolerated and to reduce blood Phe concentrations to clinically meaningful levels in previously exposed subjects in this study. Following completion of Study PAL-004, subjects have the option to enroll into this	

Section No./Title		
	study and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months. In this study, subjects in PAL-002 and PAL-004 will continue to be administered the same rAvPAL-PEG that was administered in PAL-002 or PAL-004. If the safety, PK, and blood Phe concentration results from either PAL-002 or PAL-004 indicate that the appropriate level of PEGylation and dosing regimen differs from that administered in PAL-002 or PAL-004, the PEGylation and/or dosing regimen administered in this study may change.	
Section 7.3/Study Rationale	Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. To date (JAN2011), there is limited information on the effects of a rAvPAL-PEG dose interruption in subjects with PKU. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments. There is no information regarding the pharmacokinetic (PK) profile of rAvPAL-PEG when blood Phe levels are at 60-600 µmol/L for subjects on a multiple-dose regimen. To address this lack of information, additional PK sampling will be performed prior to the dosing interruption. Upon restarting rAvPAL-PEG, PK sampling will also be performed to allow for collection of drug exposure data (area under the plasma concentration-time curve [AUC] and maximum plasma concentration [C_{max}]), absorption rate, and clearance for subjects who have been previously exposed to rAvPAL-PEG in this Substudy. This information will	1, 5

Section No./Title		
	allow for comparisons to be performed with subjects who were naïve to previous rAvPAL-PEG treatment from the Phase 1 study, PAL-001. Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationsihp of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary. This study is an extension of the dose-finding studies~~y~~ (PAL-002 and PAL-004). Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.	
Section 7.4/Summary of Overall Risks and Benefits	It is not expected that data from PAL-002 or PAL-004 will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use.	4
Section 7.4.1/Toxicitity Due to Exposure to PEG	Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg	25

Section No./Title		
	rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).	
Section 7.4.2/Toxicity Due to an Immunologic Reactions	In Study PAL-001, there were 11 subjects (out of 25 subjects in the study) with skin-related reactions (injection-site bruising, erythema, pain, rash, swelling, and urticaria) that occurred following the single administration of rAvPAL-PEG. For all of these subjects, the reactions were generalized skin reactions or injection-site skin reactions that did not compromise other organs and were not life-threatening. These suspected antibody-mediated responses were nonserious and mild or moderate in severity. These subjects are not considered to be at significant risk for antibody-mediated reactions with injection of rAvPAL-PEG during PAL-002, PAL-004, or this study; however, specific precautions for these subjects will be taken during this study to monitor subject safety (refer to Section 9.1.1). In Study PAL-002, 33 subjects have been dosed to date (JAN2011), including at dose levels of 2.0 mg/kg/week (or 0.4 mg/kg/5 days a week). Most reactions reported in these subjects have been nonserious and mild or moderate in severity. There has been 1 SAE reported to date; the event was reported as not related to treatment with study drug. There have been no reports of anaphylaxis to date (JAN2011). Subjects who have a systemic reaction during this study will undergo a series of assessments to monitor safety, including assessment of IgE antibodies, prior to determining if dosing may resume. Additionally, subjects who have systemic reactions will be premedicated and will be monitored closely for safety (refer to Section 9.1.6 for additional information regarding continued treatment of subjects with systemic reactions during the study).	9, 11

Section No./Title		
Section 7.4.2.1/Systemic Skin Reactions	Two out of 25 subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. ~~Both of these events were reported as serious and followed administration~~ One of these events was reported as serious, and the other was reported as nonserious; both events were reported following administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications. The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the [redacted]. Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. ~~The~~ Upon study completion, the subject continue~~d~~s to receive regular doses (every 3 months) of Depo-Provera, and no further reactions ~~have been~~were reported ~~to~~ date (15OCT2009). The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an	11

Section No./Title		
	allergist. See Section 7.2.1 for the results from Study PAL-001. The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. ~~It is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG.~~ The results from nonclinical studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys indicated the formation of anti-rAvPAL antibody titers that did not correspond with observations of injection-site reactions. Quantitation of anti-PEG titers were not assessed in the chronic repeat-dose studies in the rat and monkey. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to, during, and after this study is prohibited as a precautionary measure (refer to Section 9.3.2 and Section 9.4.8.).	
Section 7.4.2.2/Management of Allergic Reactions	Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of ~~2~~30-60 minutes~~hours~~ post-injection. Longer observations may be required at the discretion of the PI. The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the	21

Section No./Title		
	rAvPAL-PEG injection. Subjects who qualify for self administration of study drug will be provided with emergency resuscitation instructions (refer to Section 9.4 and the Subject Self-Administration Training Materials). In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for antibody testing. This additional testing may occur up to 6 months following the final study visit. The following measures are recommended for the treatment of allergic symptoms: • ~~Clearing~~Maintain the airway.	
Section 7.4.3/Effects of Stopping and Re-Starting rAvPAL-PEG Treatment	The effects of starting, stopping, and re-starting rAvPAL-PEG dosing on safety and blood Phe reductions previously achieved through rAvPAL-PEG dosing are not well defined, and there is limited clinical experience with rAvPAL-PEG. Allergic reactions are a common and expected clinical outcome of re-starting biologic drugs, such as rAvPAL-PEG, and may occur when subjects re-start rAvPAL-PEG dosing. However, no information reported from the ongoing clinical studies, including this study, or nonclinical results point to an increased safety risk *or decreased efficacy when rAvPAL-PEG dosing is stopped and then re-started at the same dose level:* • Nonclinical experience in the *the* BTBR *Pah*^{enu2} (ENU2) mouse model indicates that dosing interruptions of 2-4 weeks for mice administered rAvPAL-PEG 80 mg/kg/week had no increased safety risk upon re-starting dosing and there was an immediate return to stable blood Phe reductions (refer to Section 7.1). • Antibody results from the ongoing Phase 2 study, PAL-002, and this ongoing study to date (JAN2011) have not indicated an immune response that points to an	1

Section No./Title		
	increased safety risk or decreased efficacy upon re-introduction of drug. There has not been any immediate immunoglobulin E (IgE)-mediated reactions to rAvPAL-PEG observed following administration of a rAvPAL-PEG injection. All reactions to rAvPAL-PEG have been reported approximately 12-24 hours following administration. To date, 1 out of 33 subjects has tested positive by serology to IgE antibodies without reporting any clinical symptoms to suggest anaphylaxis or a severe systemic reaction. Thus, it is not anticipated that reactions to rAvPAL-PEG are IgE-mediated. • To date (JAN2011), the clinical experience with re-starting rAvPAL-PEG dosing for subjects from Study PAL-001 who rolled over into Study PAL-002 does not indicate an increased safety risk or a decreased effect on reducing blood Phe levels upon re-introduction of drug. o Nine subjects who received a single dose of rAvPAL-PEG and completed the Phase 1 study, PAL-001, rolled over into the multiple-dose, Phase 2 study, PAL-002. After a period of several months during which no drug was administered, these 9 subjects re-started rAvPAL-PEG dosing in Study PAL-002 at the same starting doses as was administered in Study PAL-001. There has been no difference to date in the safety profile or efficacy profile of the subjects from Study PAL-001 who rolled over into Study PAL-002 (previously exposed to rAvPAL-PEG) when compared with subjects who were naive to rAvPAL-PEG dosing in Study PAL-002. Although previously exposed subjects had higher and a faster return of anti-PAL and anti-PEG IgG titers than those of naïve subjects, these results have not been associated with a higher safety risk or efficacy riskbased on information to date.	

Section No./Title		
	○ In Study PAL-002, 4 subjects to date have had a protocol-defined (per Protocol Amendment # 3 dated 30APR2010), 2-week washout (no dose administered) following several weeks of a fixed dose of 0.06 mg/kg/week or 0.1 mg/kg/week rAvPAL-PEG. Following the 2 weeks where no dose was administered, all 4 subjects re-started rAvPAL-PEG dosing at a higher dose with no safety issues reported to date. Blood Phe levels for these 4 subjects have not changed upon re-introduction of dosing. ○ Clinical experience with subjects who have had to temporarily halt dosing due to an adverse event also provides information that stopping and re-starting rAvPAL-PEG dosing does not appear to increase the risk to subject safety. To date, 10 subjects participating in either PAL-002 or PAL-003 have had to temporarily stop dosing due to an adverse event. All 10 subjects resumed dosing following resolution of the event or per determination by the Investigator that subject safety would not be jeopardized with re-starting dosing. These 10 subjects resumed dosing at a reduced dose level after approximately 1-7 weeks of no dosing. Upon re-starting dosing, none of these 10 subjects have experienced additional safety issues. Eight subjects continue to receive dosing with rAvPAL-PEG (APR 2011) and have subsequently increased their dose level without additional reported adverse events that have resulted in a subsequent dosing interruption. In this study, measures will be taken to reduce the risk to subject safety upon re-starting rAvPAL-PEG dosing while still allowing for reductions in blood Phe level to clinical significance (blood Phe reduction to 60-600 µmol/L). To mitigate the possible safety risks	

Section No./Title		
	upon re-starting rAvPAL-PEG dosing, re-introduction of dosing should be performed in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call should be made to subjects 24 hours following re-starting of dosing to monitor subject safety. Subjects will also continue to be assessed per the safety assessment and stopping criteria defined in Section 9.1.3 and Section 9.1.4, and dose decreases will continue to be performed relative to safety as defined in Section 9.1.2.2. To mitigate the possible reduced effect on blood Phe level upon re-starting rAvPAL-PEG, subjects in this study who interrupt dosing whether per the protocol (ie, subjects participating in the Substudy; refer to Section 9.1) or for other reasons (refer to Section 9.4.10) will re-start rAvPAL-PEG dosing at the same dose level.	
~~Section 7.4.3/Effects of Low Blood Phe~~	~~Insufficient Phe intake or excessive rAvPAL-PEG exposure may cause blood Phe concentrations to be too low (eg, < 30 µmol/L). Prolonged low blood Phe concentrations can result in a catabolic state associated with poor growth and altered body functions, including mental and physical alterations, loss of appetite, anemia, rashes, and diarrhea. To ensure safety during this study, subjects will be monitored closely with frequent blood Phe concentration determinations. If a subject's blood Phe concentration is too low, the subject's diet, current dose of study drug, or both may be adjusted.~~	9
Section 8/Study Objectives	The secondary objectives of the study are: • To evaluate the immune ~~antibody~~ response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU. • ~~To evaluate steady-state PK of rAvPAL-PEG in subjects with PKU.~~ The exploratory objective of the study is as follows: • To assess the long-term relationship of diet and change in blood Phe concentration	1, 5, 14

Section No./Title		
	following administration with rAvPAL-PEG in subjects with PKU. The Substudy objectives are as follows: • To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU. • To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU. • To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU. • To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.	
Section 9.1/Overall Study Design and Plan	This is a long-term extension of ~~a~~thePhase 2, open-label, dose-finding studies,~~y (Study~~ PAL-002 and PAL-004, in ~~35-50~~ approximately 1000 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5~~1~~.0 mg/kg/week~~per injection~~), provided no dose-limiting toxicity i~~was~~ observed in PAL-002 or PAL-004. Only subjects who completed the protocol-defined study drug regimen ~~participation~~ in PAL-002 or PAL-004 will be enrolled into this study. Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration due to restarting dosing in this study. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.	1-4, 6, 27

Section No./Title		
	Diet ~~will~~ should not be altered during the course of this study~~, except as necessary for safety as determined by blood Phe concentrations. If a subject's blood Phe concentration is < 120 µmol/L, the subject's diet may be adjusted to increase the amount of dietary Phe intake to maintain target blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. For information regarding changes to diet for safety and decreases in dose, refer to Section 9.1.2.2.~~ Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigaor in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff. Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided. Subjects must meet a set of criteria to be considered appropriate for self administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self administration. Additional information is provided in the Subject Self-Administration Training Materials. Subjects will be evaluated for safety ~~and for blood Phe concentrations~~ throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4~~3~~. ~~At least one interim analysis may be performed by the sponsor during the study.~~ In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. PAL-002 and PAL-004 subjects' rAvPAL-PEG dosing will continue in PAL-003~~without interruption of dosing~~. In PAL-003, each subject's dose will be adjusted as	

Section No./Title		
	needed to attain or maintain blood Phe concentrations of 6~~12~~0-600 µmol/L. Doses will be evaluated on an individual basis. Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 2.1.1, Table 2.1.2, Table 2.1.3, and Table 2.1.4 (Schedule of Events). After the subject's blood Phe concentration has been controlled to within a target range (6~~12~~0-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.~~but not to exceed 3x/week (fingerstick tests may be performed more frequently).~~ A subject will continue in PAL-003 until one of the following occurs: 1. The subject withdraws consent and discontinues from the study. 2. The subject is discontinued from the study at the discretion of the Investigator. 3. The subject has completed the study through the Month ~~24~~60 visit. 4. The study is terminated. Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety. A subset of up to 10 subjects who are already enrolled and who have consented to participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they	

Section No./Title		
	also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption. To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.	
Section 9.1.1/Management of Local and Systemic Reactions to rAvPAL-PEG	Subjects who qualify for self administration of study drug will be provided with information and instruction with regard to management of local and systemic reactions (refer to the Subject Self-Administration Training Materials).	11, 13, 21
Section 9.1.1.1/Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-	Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from PAL-002 or PAL-004 are excluded from participation in this study. Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in	11, 13, 21

Section No./Title		
containing Product	PAL-002 or PAL-004are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or antihistamines 1 hour prior to study drug dosing for the remainder of the study. The premedication dosage will be standard. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable). Subjects may develop systemic, large local skin, or local skin reactions after enrollment in PAL-003. Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.	
Section 9.1.1.2/Definitions of Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously	• Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological; and/or a fever attributed to treatment with rAvPAL-PEG (≥ 100.4°F or ≥ 38°C).	11, 13, 21
[redacted]	[redacted]	[redacted]
Section 9.1.3.3.1/Local Skin Reactions	Subjects who have had a local skin reaction (refer to Section 9.1.1.2 for definition) after administration of rAvPAL-PEG in ~~PAL-001, PAL-002,or~~ this study may continue if the symptoms do not worsen and no other symptoms have developed but may be premedicated with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug dosing. ~~Recommended premedications are~~	11, 13, 21

Section No./Title		
	~~acetaminophen and antihistamine (Zyrtec [cetirizine] is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Zyrtec or 25 to 50 mg oral Benadryl [diphenahydramine]).~~ Because antihistamines ~~Benadryl~~ can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from ~~the~~ study treatment~~and complete an Early Termination Visit~~.	
Figure 9.1.1.3.2/Large Local Skin Reactions	Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects may be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing. ~~The location of the large local skin reaction relative to the rAvPAL-PEG injection site will determine if a subject may remain in the study and continue to be administered rAvPAL-PEG. An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.~~ ~~Subjects who have large local skin reactions that are contiguous to the injection site may remain in the study. Subjects who have large local skin reactions that are not contiguous to the injection site may have additional assessments performed (Unscheduled~~	11, 13, 21

Section No./Title		
	~~Hypersensitivity Reaction Visit) per Investigator discretion and must then be terminated from the study. A decision to terminate a subject from the study must be agreed to by both the Investigator and the Sponsor's Medical Officer.~~	
Section 9.1.1.3.2.1/Large Local Skin Reactions Contiguous to Injection Site	Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. Large local skin reactions that are contiguous to the injection site should be managed as local skin reactions (refer to Section 9.1.1.3.1). For the remainder of the study, subjects ~~must~~ may be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug ~~dosing~~. ~~Recommended premedications are acetaminophen and antihistamine (Zyrtec [cetirizine] is preferred). The dosage will be standard (eg, 1000 mg acetaminophen and 10 mg Zyrtec or 25 to 50 mg Benadryl [diphenahydramine]).~~ Because antihistamines~~Benadryl~~ can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).	11, 13, 21
Section 9.1.1.3.2.2/Large Local Skin Reactions Not Contiguous to Injection Site	Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction (refer to Section 9.1.1.3.3). An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk. ~~Subjects who develop large local skin reactions that are not contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may not continue to~~	11, 13, 21

Section No./Title		
	~~participate in the study. Subjects may have additional assessments performed (ie, Unscheduled Hypersensitivity Skin Reaction Visit) immediately after the reaction (within 48 hours): serum anti-rAvPAL-PEG antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL immunoglobulin M [IgM], anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG immunoglobulin E [IgE]); serum tryptase level; sedimentation rate; C-reactive protein (CRP), total hemolytic complement (CH50), and complements C_1 and C_4; and clinical laboratory tests (urinalysis, chemistry, hematology). Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4).~~	
Section 9.1.1.3.3/Systemic Reactions	Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG must stop further administrations of rAvPAL-PEG and must immediately return to the clinic for safety assessments ~~will be terminated from the study~~. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 48 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity ~~Skin~~ Reaction Visit): serum anti-rAvPAL-PEG antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibody)~~anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE~~); serum tryptase level (~~optional;~~ it is recommended that this sample be drawn immediately after reaction); sedimentation rate; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1). Following completion of the Unscheduled Systemic Reaction Visit, administration of	11, 13, 21

Section No./Title		
	rAvPAL-PEG may resume up to 1 week following the onset of the reaction if the systemic reaction is not IgE-mediated and the subject's safety will not be further compromised with resumed dosing. rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects must be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable). If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section 9.1.2. Subjects must be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable). ~~Subjects who have a systemic reaction without generalized skin symptoms may have these assessments performed per Investigator discretion. Subjects will then be terminated from the study and should be scheduled to complete the Early Termination Visit (refer to Section 12.4).~~	

Section No./Title		
Section 9.1.2/Dose Modifications	~~After any modifications (increases or decreases) to dose level or frequency, subjects should remain in the clinic for observation for at least 2 hours following the injection of study drug. The Investigator should remain at the site to assess the subject for safety.~~	2, 3, 21, 22, 23
Section 9.1.2.1/Dose Increase Methodology	Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Study Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations. ~~Blood Phe levels will be measured 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.~~ For subjects from PAL-002~~Depending upon the response to rAvPAL PEG in PAL-002~~, an individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows. • The dose may be increased by increments of no more than 10-fold higher than the subject's current dose. • When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, ~~and~~ 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg) ~~that were defined in the previous study (PAL-002).~~or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) and drug concentrations, as well as any adverse events (any hypersensitivity reaction) for an individual subject. • The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5~~2~~.0 mg/kg~~, including subjects who receive more than 1 dose/week.~~ Subjects who increase their dose frequency should perform the Interim Dosing Visit assessments (refer to Section 12.3.6~~5~~ and Table 2.1.3 ~~Table 2.1.3:~~ and Table 2.1.4 ~~Table 2.1.4:~~).	2, 3, 21, 22, 23

Section No./Title		
	• When a dose is increased ~~(either by increasing the dose level or by increasing the frequency of dosing)~~, the subject must ~~remain in the clinic~~be observed for ~~a minimum of~~ 30-60 minutes~~2 hours~~ following the first modified dose administration~~, and an Investigator will remain on site during this time~~.Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount. • Only 1 dose (level or frequency) adjustment is allowed every 2 weeks. • Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.	
Section 9.1.2.2/Dose Decrease Methodology	~~A subject's diet should be assessed prior to any dose decreases. Changes to diet should only be made if a subject's blood Phe concentrations < 120 µmol/L. If a subject's blood Phe level is < 120 µmol/L, the subject's dietary Phe intake should be increased to achieve a stable blood Phe concentration of 120-600 µmol/L. If the subject's blood Phe level is < 120 µmol/L despite a high Phe diet, the rAvPAL-PEG dose will then be decreased. The subject will be asked about any changes to diet prior to any dose adjustments, and any changes to diet should be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.~~ All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L~~> 30% change from baseline~~) ~~and drug concentrations~~, as well as any adverse events~~toxicity~~ (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for safety (ie, ~~blood Phe concentration < 120 µmol/L) or other toxicity~~any adverse event that may be improved with a lower, more frequent dose). A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between	2, 3, 21, 22, 23

Section No./Title		
	these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.	
Section 9.1.3/Safety Assessment ~~Stopping~~ Criteria	If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Study Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.	8
Section 9.1.4/Stopping Criteria	If~~n addition, if~~ 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will be evaluated and ~~halted and no subject will be treated at a higher dose level until~~ a safety assessment will be ~~is~~ completed. In addition, the Food and Drug Administration (FDA) may ~~will~~ be notified of this occurrence if appropriate.	8
Section 9.3.1/Inclusion Criteria	Individuals eligible to participate in this study must meet all of the following criteria: 1. Must have completed participation and all protocol-defined study drug in PAL-002 or PAL-004.	4
Section 9.3.2/Exclusion Criteria	Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 1. Use of any investigational product (with the exception of rAvPAL-PEG) or	4, 11

Section No./Title		
	investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments. 2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug. 3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation. 4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction of generalized rash may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study. 9. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from PAL-002 or PAL-004.	
Section 9.3.3/Removal of Subjects from Treatment or Assessment	Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study treatment should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit	8, 27

Section No./Title		
	should be carried out (refer to Section 12.4). BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance. Reasons for which the Investigator or BioMarin may withdraw a subject from the study treatment include, but are not limited to, the following: • Subject experiences a serious or intolerable adverse event (AE). • Subject develops a clinically significant laboratory abnormality. • Subject requires medication prohibited by the protocol. • Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy). Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following: • Subject does not adhere to study requirements specified in the protocol. • Subject was erroneously admitted into the study or does not meet entry criteria. • Subject is lost to follow-up. • ~~Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).~~	
Section 9.3.4/Subject Identification and	Subjects will retain the same subject number assigned in PAL-002 or PAL-004.	4

Section No./Title		
Replacement of Subjects		
Section 9. 4/Treatments	Upon enrollment into this study, subjects from PAL-002 will be dosed with the same or higher dose that was administered upon completion of PAL-002. Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed to by the PI and the Study Medical Officer, based upon the subject's response to doses. Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided. Subjects may be eligible to self administer study drug if he or she meets the following criteria: • The subject is on a stable dosing regimen for 2 weeks. • The subject has not experienced any CTCAE Grade 3 or higher adverse event. • The subject has not experienced any hypersensitivity reaction to rAvPAL-PEG for at least 4 weeks. • The subject has no cognitive impairments that may increase the safety risk of self administration per the assessment of the PI. • The subject has been approved for self administration of study drug by the	10

Section No./Title		
	Sponsor's Medical Officer. • The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely. • The subject has been provided with epinephrine and has been trained on when and how to administer it. Qualified study site personnel will train each eligible subject on all procedures for self administration of study drug under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self administer a dose at home. The patient will see a study site nurse or home healthcare nurse in person every week to ensure that the subject continues to perform all self-administration procedures correctly, to assess for adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 2.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials. Subjects who are eligible for self administration will be provided with a Subject Study Manual, Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following: • How to prepare and perform the injections of study drug safely. • How to receive, track, store, prepare, and return both used and unused study drug.	

Section No./Title		
	• How to safely use and dispose of syringes used for injections of study drug. • How to care for their injection site after an injection of study drug. • How to identify an adverse reaction and how to report this reaction to the study site. • How and when to use epinephrine. • Who to contact at the study site in case of an emergency. The PI or the Sponsor's Medical Officer may request that self administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.	
Section 9.4.1/Treatments Administered	BioMarin and/or its designee will provide the study site with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplyingthe study drug to subjects who qualify for self administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites. Information regarding supplying study drug to subjects who qualify for self administration is provided in the Subject Self-Administration Training Materials.	10, 27
Section 9.4.3/Storage	~~At the study site, all~~ IP must be stored at 5 ±3°C (41 ± 5°F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.	10, 27

Section No./Title		
	Specific instructions for storage of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.	
Section 9.4.4/Directions for Administration	Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Study Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0~~2~~ mg/kg. Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1. Information for dosing of subjects who enroll into this study after completing Study PAL-004 is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10. Instructions for administration of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may~~will~~ need to be re-evaluated (refer to Section 9.4.10).	2, 10, 27
Section 9.4.5/Method of Assigning Subjects to Treatment Groups	Subjects will retain the same subject number used in PAL-002 or PAL-004.	4, 27

Section No./Title		
Section 9.4.6/Selection of Doses Used in the Study	This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 6~~12~~0-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study and the ongoing study, PAL-002, to date (JAN2011). ~~The doses for this study are based upon the results of nonclinical studies and PAL-001. The no observable adverse effect level (NOAELs) from nonclinical studies in rats and monkeys represents a greater than 100-fold safety factor over the starting dose administered in PAL-002 (0.001 mg/kg).~~	3
Section 9.4.6.1/Selection of Timing of Dose for Each Subject	Study drug will be administered ~~in the morning~~ by clinic staff or other qualified and trained study personnel. Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided (refer to Section 9.4).	19
Section 9.4.8/Prior and Concomitant Medications	Use of any investigational product (with the exception of rAvPAL-PEG used in PAL-002 or PAL-004) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a	4, 11

Section No./Title		
	PEG-containing product will be premedicated with acetaminophen and/or antihistamines (refer to Section 9.1.1). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Study Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit. Subjects who have had a prior systemic reaction to rAvPAL-PEG or a PEG-containing product ~~are excluded from~~may participat~~ion~~ in this study (refer to Section 9.1.1).	
Section 9.4.10/Dose Interruption and Missed Doses	Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1. Information for dosing of subjects who enroll into this study after completing Study PAL-002 or Study PAL-004 is provided in Section 9.1. During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator in consultation with the Sponsor's Medical Officer. Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 2.1.1, Table 2.1.2, Table 2.1.3, and Table 2.1.4) during any dosing interruption.	7

Section No./Title		
	Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study treatment per discretion of the Sponsor or Investigator (refer to Section 9.3.3).	
Section 9.5/Investigational Product Accountability	The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.	27
Section 9.5.1/Return and Disposition of Clinical Supplies	Subjects who qualify for self administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).	10
Section 9.6/Dietary or Other Protocol Restrictions	Subjects will be instructed that diet should not be altered during the course of the study~~. except as necessary for safety. If a subject's blood Phe concentration is < 120 µmol/L, the subject's diet will be adjusted to maintain blood Phe concentrations of 120-600 µmol/L. Changes to diet will be made per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.~~ Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigaor in consultation with the Sponsor's Medical Officer. A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next dose of study drug. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.	6

Section No./Title		
Section 9.7.1.5/Chest X-Ray	A chest X-ray will be performed at the Screening, Month 12, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.	24
Section 9.7.2.1/Blood Phenylalanine Concentrations	Blood samples for Phe concentration measurements will be drawn ~~in the morning,~~ at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 2.1.1, Table 2.1.2, Table 2.1.3, and Table 2.1.4). In addition, after each administration of study drug, the subject will have a blood Phe measurement by fingerstick as outlined in Table 2.1.1, Table 2.1.2, Table 2.1.3, and Table 2.1.4. This may be done by the subject at home. ~~Subjects who have their dose frequency increased will have additional Phe assessments performed as outlined in Table 2.1.3: and Table 2.1.4: .~~	19, 27
Section 9.7.3/Pharmacokinetic Variables	~~Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentration (120-600 µmol/L) for a minimum of 2 weeks (to allow assessment of blood Phe concentrations) and no further dose modification is planned. Once this target has been achieved, PK samples will be collected predose on the same day each week for 3 consecutive weeks.~~ Subjects enrolled in the Substudy will have additional PK sampling performed prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG. For subjects participating in the Substudy, PK sampling will be performed as follows:	1, 14

Section No./Title		
	• When dosing is stopped, PK sampling will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16, hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours. • When dosing is re-started, PK sampling will be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours. ~~Subjects who have increased their dose frequency to twice weekly or daily will have addition PK assessments performed for 5 weeks after the change in dose regimen (refer to Section 12.3.5 and Table 2.1.3: and Table 2.1.4:).~~ BioMarin will perform the analysis.	
Section 9.7.4/Exploratory Efficacy Variable	Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each week. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.	5
Section 9.7.5.2/Antibody Testing	Immunogenicity will be assessed by determining ~~antibody~~immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1.3.3).~~1) anti-rAvPAL immunoglobulin G (IgG) antibodies, 2) anti-rAvPAL immunoglobulin M (IgM) antibodies, 3) anti-PEG IgG antibodies, 4) anti-PEG IgM antibodies, 5) anti-rAvPAL-PEG immunoglobulin E (IgE) antibodies, and 6) anti-rAvPAL-PEG Enzyme Activity Neutralizing antibodies (NAb), and 7) rAvPAL~~	23, 27

Section No./Title		
	~~PEG antibody clearing complexes (if needed).~~ Validated immunogenicity assays will be used per the time points indicated in the Schedule of Events (Table 2.1.1 and Table 2.1.2). BioMarin will perform the analysis except for ~~anti rAvPAL PEG~~ IgE antibodies, which will be assessed by a Contract Research Organization.	
Section 9.7.6/Clninical Laboratory Assessments	All clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not any abnormal ~~the~~ result is clinically significant. Table 9.7.6.1: Clinical Laboratory Tests has been revised to include information for subjects who have a positive result from the urine pregnancy test.	27
Section 10.1/ Adverse Events	~~According to the ICH definition, an AE (or adverse experience) is "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the IP."~~ For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a patient administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following: • AEs not previously observed in the subject that emerge during the course of the study. • Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study. • Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-	15

Section No./Title		
	in). ~~An adverse drug reaction (ADR) is described by the ICH as "all noxious and unintended responses to a medicinal product related to any dose." This means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, ie, the relationship cannot be ruled out~~An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the study-drug and the AE. ~~An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma).~~ Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis. ~~The reporting period for non-serious AEs is the period from the first administration of study drug through the final F/U Visit or at the ETV. If a non-serious AE remains unresolved at the conclusion of the study, the PI and medical monitor will make a joint clinical assessment as to whether continued follow-up of the AE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.~~ The study period during which all non-serious AEs and SAEs will be reported begins after informed consent is obtained and the first adminstration of study drug and continues until 4 weeks following the last administration of study drug or the last visit of the treatment period (refer to Section 12). After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. The criteria for determining, and the reporting of SAEs is provided in~~The Investigator will assess AEs for severity, for relationship to IP, and as to whether the event meets one or more of the definitions of a serious adverse event (SAE; refer to~~ Section 10.2~~)~~.	

Section No./Title		
	The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. Resolution of AEs (with dates) should be documented on the appropriate CRF page(s) and in the subject's medical record. The Investigator responsible for the care of the patient or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.~~and will record it on the source documents and AE CRF, using the CTCAE v 3 grades defined below. Events that are CTCAE grades 4 and 5 are serious events and require completion of both an SAE form and AE eCRF.~~	

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]

Section No./Title		
	3 — Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL) 4 — Life threatening or debilitating: consequences; urgent intervention indicated[b] 5 — Death related to AE Grade 4 and 5 AEs should always be reported as SAEs. [a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money. [b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden. ~~The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.~~ The Investigator will determine the relationship of an AE to the ~~IP~~study drug and will record it on the source documents and AE CRF, using the categories defined below.	
Section 10.2/Serious Adverse Events	A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:	15

Section No./Title		
	• Is fatal. • Is life threatening. Note: Life-threatening refers to an event that places the patient at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death. • Requires or prolongs inpatient hospitalization. • Results in persistent or significant disability or incapacity. • Is a congenital anomaly or birth defect in the child or fetus of a patient exposed to IP prior to conception or during pregnancy. • Is an important medical event or reaction. ~~A SAE is defined as any AE that:~~ ~~• Results in death.~~ ~~• Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.~~ ~~• Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization. Admission of a subject to the hospital as an in-patient as a result of an AE, even if the subject is released on the same day, qualifies as hospitalization.~~ ~~• Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.~~ ~~• Is a congenital anomaly or birth defect, that is, an AE that occurs in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.~~	

Section No./Title		
	~~• Is an important medical event that does not meet any of the above criteria, but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.~~ ~~More than one of the above criteria may apply to any specific event.~~ The reporting period for SAEs begins after informed consent is obtained, and continues until 4 weeks following the last administration of study drug or End of Treatment Visit.~~The reporting period for SAEs begins earlier than non-serious AEs and is the period from the time of signing of the ICF~~ through 4 weeks after the last dose or at the ETV. ~~SAEs reported to the Investigator outside of this reporting period will be reported to BioMarin if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, even if this extends beyond the study reporting period. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.~~ ~~Any SAE, whether or not considered related to study drug, will be reported immediately (within 24 hours) to BioMarin Pharmacovigilance by fax using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate CRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying BioMarin Pharmacovigilance of an SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by BioMarin as soon as it becomes available.~~ All SAEs, whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form to BioMarin	

Section No./Title		
	Pharmacovigilance (BPV).). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the patient is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the patient's medical record. For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report). At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment. The investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.	

Section No./Title		
Section 10.3/Pregnancy	Pregnancy in a subject or partner should be reported ~~immediately (~~within 24 hours of the site becoming aware of the pregnancy~~)~~ to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.	15
Section 10.4/Urgent Safety Measures	The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours. Examples of situations that may require urgent safety measures include discovery of the	15

Section No./Title		
	following: • Immediate need to revise the IP administration (ie, modified dose amount or frequency not defined in protocol). • Lack of study scientific value, or detrimental study conduct or management. • Discovery that the quality or safety of the IP does not meet established safety requirements.	
Section 11.2/Diet Diary	A 3-day diet diary will be issued to subjects for completion and will be brought to each clinic visit for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.	5
Section 12.2/Screening Visit	• Chest X-ray For subjects who participated in PAL-002 or PAL-004, these assessments may be the same as those used for the Week 16 Visit of PAL-002 or the Week 16 visit of PAL-004, if they occur within 28 days from Day 1.	24, 26
Section 12.3/Treatment Period	During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done	26

Section No./Title		
	predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done ~~on the third day~~daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.	
Section 12.3.1/Day 1 (Week 1)	• 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.) • ~~Predose~~ PK sample • ~~Predose b~~Blood Phe and plasma tyrosine concentration • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~ • Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer) o For subjects enrolling into this study from Study PAL-004, first dose of rAvPAl-pEG must be given in the clinic setting and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.	26

Section No./Title		
Section 12.3.2/Weekly Visits	Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. However, dose modifications must be peformed in the clinic. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. • ~~Predose~~ PK sample o For subjects participating in the Substudy, refer to Section 12.3.3. o ~~Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.~~ • ~~Predose b~~Blood Phe and plasma tyrosine concentration o ~~Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.~~ • Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer) o For subjects participating in the Substudy, refer to Section 12.3.3.	26

Section No./Title		
Section 12.3.3/Substudy Visits (Weekly)	The following study activities will be performed on Day 1 of the Substudy (ie, final dose prior to interruption), Day 8, Day 15, Day 22, and Day 29 (ie, restart administration of study drug) for subjects participating in the Substudy: • Physical examination • Vital signs, including weight • Clinical laboratory tests • Sedimentation rate • Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) • Injection-site inspection (predose; postdose on Day 1; during weekly visit on Day 8, 15, 22; and postdose on Day 29) • Assessment of AEs • Concomitant medications • Diet query • 3-day diet diary • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies,) • Blood Phe and plasma tyrosine concentration o When dosing is stopped, sampling for blood Phe assessment only will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16 hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours. o When dosing is re-started, sampling for blood Phe assessment only will	1, 26

Section No./Title		
	be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours. • PK sample o For subjects participating in the Substudy, additional PK sampling will be performed upon the final dose prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG. Refer to Section 9.7.3 • Administer study drug (Day 29 only) o For subjects participating in the Substudy, administration of rAvPAL-PEG will not be performed for approximately 4 consecutive weeks regardless of their dosing regimen (dose level and frequency). After approximately 4 weeks of no dosing, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of re-administration must be given in the clinic setting, and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.	
Section 12.3.4/Monthly Visits (Week 4, 8, 12, etc)	Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. Monthly visits consist of all weekly activities and include additional activities and must be performed in the clinic. The following study activities will be performed at the monthly visits beginning with Week 4: • ~~Predose~~ PK sample ~~(subjects who are on a stable dosing regimen for ≥ 3 months~~	26

Section No./Title		
	~~only)~~ o For subjects participating in the Substudy, refer to Section 12.3.3. ~~o Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.~~ • ~~Predose b~~Blood Phe and plasma tyrosine concentration ~~o Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose plasma Phe and tyrosine assessments may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. The weekly plasma Phe assessment may be replaced with a Phe fingerstick test. Weekly testing should resume if the subject's dosing regimen changes.~~ • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~ • Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer) o For subjects participating in the Substudy, refer to Section 12.3.3.	

Section No./Title		
Section 12.3.5/Quarterly Visits (Week 12, 24, 36, etc)	Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. • Predose PK sample ~~(subjects who are on a stable dosing regimen for ≥ 3 months only)~~ o For subjects participating in the Substudy, refer to Section 12.3.3. ~~o Per investigator discretion, once a subject is on a stable dosing regimen for at least 3 months (as evidenced by blood Phe levels of 120-600 µmol/L for the majority of the 3 months), the predose PK assessment may be skipped at the weekly visits and performed at the monthly visits for the first year then 2-3 months thereafter. Weekly sampling should resume if the subject's dosing regimen changes.~~ • ~~Predose b~~Blood Phe and plasma tyrosine concentration • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~ • Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer) o For subjects participating in the Substudy, refer to Section 12.3.3.	26
Section 12.3.6~~5~~/Interim Dosing Visit	~~• PK sample~~ ~~o Subjects increase their dose frequency to 2x/week will have an additional predose PK sample obtained prior to administration of the first 4 doses (ie, for 2 weeks after the dose frequency increase). For the remaining 3 weeks after~~	26

Section No./Title		
	~~the dose frequency increase, a PK sample will be obtained weekly prior to the first dose. Refer to Table 2.1.3: for the Interim Dosing Visit schedule.~~ ~~o Subjects who have their dose frequency increased to daily will have additional PK samples obtained every day for the first week: 12 hours after the first daily dose and predose every day for the rest of the week. After the first week of daily dosing, subjects will have weekly PK samples obtained for 4 additional weeks. Refer to Table 2.1.4: for the Interim Dosing Visit schedule.~~	
Section 12.3.7/Unscheduled Hypersensitivity Reaction Visit	Subjects who have a systemic reaction, including a generalized skin reaction, or a large local skin reaction that is not contiguous to the injection site after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 48 hours) the reaction. ~~Subjects who have other systemic symptoms or a large local skin reaction that is not contiguous to the injection site (refer to Section 9.1.1) may have these assessments performed per investigator discretion.~~ • CRP, CH50, C1, C3, and C4 • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~ • Serum tryptase level o Perform immediately after reaction if possible. ~~o Assessment is optional for subjects who have a generalized skin reaction per investigator discretion.~~ • Skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction)	11, 21, 26

Section No./Title		
	Following completion of the Unscheduled Hypersensitivity Reaction Visit assessments, the subject may resume treatment with study drug per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. Subjects who have had an IgE-mediated reaction must be discontinued from further study drug administration. Subjects who do not have an IgE-mediated reaction may resume study drug administration under close monitoring; subjects must be premedicated orally with acetaminophen and/or antihistamines 1 hour prior to study drug dosing for the remainder of the study (refer to Section 9.1.5.3.3). ~~Subjects should then return to the clinic 4 weeks after their last dose of rAvPAL-PEG for the Early Termination Visit assessments (refer to Section 12.4).~~	
Section 12.4/Early Termination Visit	The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who terminate from study treatment early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion. ~~Subjects who complete an Unscheduled Hypersensitivity Reaction Visit (refer to Section 12.3.6) will be terminated from the study. Subjects will then return 4 weeks after the last dose of study drug for the Early Termination Visit.~~ • 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.) • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~	26

Section No./Title		
Section 12.5/Final Follow-up Visit	• 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Final Follow-up Visit.) • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies) ~~(anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibody, and anti-rAvPAL-PEG IgE)~~	26
Section 14.2/Safety Analysis	All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug.~~Safety will be evaluated on the incidence of AEs, including serious AEs (SAEs), and clinically significant changes in vital signs and laboratory test results.~~ Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseine, of vital signs, ECG results, and X-ray results will also be provided.~~The verbatim terms reported on CRFs to identify AEs will be coded using MedDRA. Treatment-emergent AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. Changes from baseline in vital signs and laboratory test results will be summarized with descriptive statistics.~~	26

Section No./Title		
Section 14.3/Pharmacokinetic Analysis	~~Data from all subjects who complete at least 2 weeks of the study will be included in the PK analysis.~~ ~~Steady-state PK (predose samples) of rAvPAL-PEG will be evaluated in PKU subjects after the subject has achieved and maintained target blood Phe concentrations (120~~60-600 ~~µmol/L) for a minimum of 2 weeks and no further dose modification is planned.~~ Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should data become available from PAL-002 or PAL-004 that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer~~but not to exceed 3x/week~~.	1
Section 14.4/ Efficacy Analysis	Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis. Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of ~~120~~60-600 µmol/L will be summarized by each scheduled time point. The relationship of dietary Phe intake (per information reported on the subject diet diary)	1, 3, 5

Section No./Title		
	and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis. Information regarding this exploratory analysis will be provided in the Statistical Analyais Plan. For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored. Information regarding the analysis will be provided in the Statistical Analyais Plan.	
Section 14.5/Determination of Sample Size	Subjects who participated in PAL-002 or PAL-004 may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.	1, 4
Section 14.7/Interim Analyses	~~At least one interim analysis may be performed by the sponsor during the study.~~ Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).	27
Section 20/References	Lapis et al., Long-term correction of PKU in the Pahenu2 mouse by mutant and chemically modified forms of Phenylalanine Ammonium Lyase. The American Society of Human Genetics, 57th Annual Meeting, 2007	27

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CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical Sciences BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Up to 60 months or until study is terminated
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011
Date of Amendment 4:	June 7, 2012

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 4

Date: June 7, 2012

RATIONALE AND SUMMARY OF CHANGES

The protocol for Study PAL-003 is being amended to make the following changes:

1. Assessment of spot urine albumin/creatinine ratio has been added at study entry for newly enrolled subjects. In addition, all subjects will now have spot urine albumin/creatinine ratio assessed monthly after study entry. Complements (C3 and C4) will also be collected and assessed at study entry and every 3 months for all subjects; if complement levels are abnormal upon assessment, sampling will be performed monthly.

Rationale for change: The urine albumin/creatinine ratio and C3 and C4 tests have been added because they are measures of potential renal injury resulting from an immunologic reaction to rAvPAL-PEG. Several subjects currently being treated with rAvPAL-PEG have developed antibodies to rAvPAL-PEG and evidence of consumption of complement. While no subject in a rAvPAL-PEG study has had clinical laboratory evidence of renal injury as of May 2012, these tests have been added to increase detection of sensitivity to renal injury and to evaluate the potential for a clinically meaningful safety signal.

2. Subjects who meet the criteria for blood Phe levels and rAvPAL-PEG dose will now have their blood Phe concentration collected monthly rather than weekly.

Rationale for change: Subjects will have their blood Phe concentration assessed monthly versus weekly if they have been on a stable dose of rAvPAL-PEG (ie, no dose change in the last 2 weeks) and have had a phenylalanine (Phe) level at or below target range (60-600 µmol/L) for 2 consecutive weeks, which is evidence that the dose-adjustment period has ended. For such subjects, assessment of blood Phe performed monthly is sufficient. However, if a subject's blood Phe increases to >600 µmol/L or decreases to ≤ 30 µmol/L for any of the monthly blood Phe draws, that subject should perform weekly blood Phe draws until the rAvPAL-PEG dose has been adjusted and the Phe level returns to within the protocol-defined target range for at least 2 weeks.

3. Information regarding hypersensitivity reactions and rAvPAL-PEG treatment, including how to treat hypersensitivity reactions, has been updated.

Rationale for change: As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in the Phase 1 (PAL-001) and Phase 2 (PAL-002 and -004) studies, including this extension study. With the information from these 70 subjects, there is a better understanding of the hypersensitivity reactions and rAvPAL-PEG treatment. Information regarding treatment with rAvPAL-PEG and subject care following a hypersensitivity reaction has been modified based on this additional information.

4. A fixed dose of rAvPAL-PEG will be administered in this study. Additionally, a higher concentration of rAvPAL-PEG (15 mg/ml vs. 10 mg/ml) will be administered. Subjects who have demonstrated efficacy (ie, Phe within target range 60-600 µmol/L) for at least two consecutive Phe assessments may convert their weight-based rAvPAL-PEG dose to a fixed dose and may also divide their weekly dose into a 7-days-a-week dosing regimen.

Rationale for change: The Phase 3 rAvPAL-PEG program will be conducted using a rAvPAL-PEG dosing concentration of 15 mg/ml, a fixed dose, and a 7-days-a-week dosing regimen. This extension study is being amended to incorporate the planned Phase 3 dosing regimen and concentration. Fixed dosing will simplify the dosing regimen to enable greater convenience for administration amongst the PKU patient population, especially for patients with significant neurocognitive dysfunction. The fixed doses proposed in this study are based on an average subject weight of 80 kg (based on the subjects in previous rAvPAL-PEG studies). Efficacy information regarding the fixed doses will be collected in this study and the Phase 2 study, 165-205. Subjects who are on a fixed-dose regimen will be monitored for safety per the safety plan outlined in the protocol. The 15 mg/ml rAvPAL-PEG formulation is more concentrated than the 10 mg/ml formulation, allowing for fewer injections per dose. It is hypothesized that hypersensitivity reactions could be related to maximum plasma concentration (C_{max}); therefore, subjects who qualify for home or self administration may divide their weekly dose for administration up to 7 days per week.

5. A pharmacokinetics (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. A corresponding PK substudy objective has been added.

Rationale for change: Subjects who are being treated with an efficacious dose of rAvPAL-PEG (ie, have received the same rAvPAL-PEG dose for at least 2 consecutive weeks) and for whom no further dose modifications are planned will qualify for the PK substudy.

Single-dose PK data have been collected from the Phase 1 study, PAL-001; however, there is little multiple-dose PK data when a stable dosing regimen has been implemented. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved and maintained Phe reduction to within the protocol-defined target range.

6. The previous PK substudy that required stopping and restarting rAvPAL-PEG dosing has been removed. No subjects were enrolled into this PK substudy.

Rationale for change: The previous PK substudy was designed to examine the effect of rAvPAL-PEG dosing interruptions on safety, efficacy, PK, and immune response. This substudy has been discontinued because it requires subjects to discontinue treatment with rAvPAL-PEG, which would halt any Phe reduction benefits that may have occurred with treatment. The corresponding substudy objectives have been removed. Unscheduled dose interruptions may still occur due to adverse events per the Investigator and the Sponsor's Medical Officer.

7. Finger stick Phe assessments have been removed.

Rationale for change: The plasma Phe samples currently implemented in the study provide sufficient assessment of blood Phe levels without subjecting subjects to the additional and painful finger stick procedure. The site staff may perform assessments of Phe levels at the local laboratory if needed.

8. Guidance for treating subjects who develop hypophenylalaninemia has been added.

Rationale for change: Although there is no evidence in the literature that low Phe is of clinical significance in adults, it is hypothesized that sustained low blood Phe is a clinical concern because Phe is an essential amino acid. Guidelines for treating subjects whose serum Phe levels reach ≤ 30 µmol/L for a minimum of 1 month have now been included in the study.

9. A blood draw has been removed for subjects who enroll into PAL-003 from a previous rAvPAL-PEG study. Subjects who have performed a blood draw upon completion of a previous rAvPAL-PEG study do not need to perform another blood draw at the Screening Visit of this study if the blood draw was performed within 28 days. Safety and efficacy information are not expected to change in the 28 days from completion of a previous rAvPAL-PEG study and the start of this study.

Rationale for change: Currently, subjects in Studies PAL-002 and PAL-004 immediately enroll into the PAL-003 extension study (ie, do not stop rAvPAL-PEG dosing). The PAL-003 protocol required a blood draw to be performed as part of screening that was

similar to the end-of-study blood draw performed for Studies PAL-002 and PAL-004. When subjects enroll over into Study PAL-003 within 28 days of completing a previous rAvPAL-PEG study, the screening blood draw is not needed. Safety and efficacy information is not expected to change in the 28 days from completion of a previous rAvPAL-PEG study and the start of this study.

10. Information regarding the management of allergic reactions has been updated.

Rationale for change: Administration of oral or IV glucocorticoids has been added as a recommended treatment.

11. The inclusion criteria have been modified to allow enrollment of subjects from a Phase 2 rAvPAL-PEG study.

Rationale for change: This change will allow enrollment of subjects who complete Study 165-205.

12. Assessment of urinalysis test results has been moved from the local laboratory to the central laboratory.

Rationale for change: This change will help standardize test results across sites.

13. Subject weight will now be measured monthly rather than weekly.

Rationale for change: Because the study is moving from weight-based dosing to a fixed dosing regimen (see amendment item #5 above), there is no need to obtain weight on a weekly basis.

14. Subject diet diaries will be assessed monthly rather than weekly.

Rationale for change: Weekly diet diary assessment provides no added benefit to safety or efficacy when compared with monthly diet diaries. Monthly diet diary assessments will provide sufficient safety and efficacy information while increasing feasibility of completion for sites and subjects.

15. Information regarding clinical rAvPAL-PEG studies has been updated.

Rationale for change: Information regarding Studies PAL-002 and -004 have been updated, and information regarding Study 165-205 has been added.

16. Administrative revisions have been made to improve clarity and consistency.

Specific revisions to the text of each section of the protocol (since the protocol amendment finalized on 4 May 2011), including the Synopsis, are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for

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when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

STUDY DESIGN AND PLAN:

This is a long-term extension of rAvPAL-PEG Phase 2 studies in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigator in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose (subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks). Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per

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the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

- A subject will continue in PAL-003 until one of the following occurs:
- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator.
- The subject has completed the study through the Month 60 visit.
- The study is terminated.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

PAL-003 Substudy

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed.

Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Sponsor's Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's

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current dose.

- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg)) or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject.
- The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg. Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit).
- When a dose is increased, the subject must be observed for 30-60 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

<u>Dose Decrease Methodology</u>

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for safety (ie, any adverse event that may be improved with a lower, more frequent dose).

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L as measured by serum Phe levels for a minimum of 1 month), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for

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PKU disease management, subject diet should be monitored by a dietician).

Safety Assessment Criteria:

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Sponsor's Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

Stopping Criteria:

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will be evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

NUMBER OF SUBJECTS PLANNED:

Approximately 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.

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5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.

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8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from a previous rAvPAL-PEG study.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg or rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose than the dose that was administered upon completion of that study provided that there was no interruption in dosing. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. . rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Sponsor's Medical Officer, based upon the subject's response to doses.

Fixed Dosing

Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive weeks) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects should convert their weight-based dose using the following table:

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 10 mg/ml concentration (ml)	Vol for 15 mg/ml concentration (ml)
0.03	2.5	0.25	0.17
0.06	5	0.5	0.33
0.12	10	1	0.67
0.25	20	2	1.33
0.5	40	4	2.67
1.0	75	7.5	5
2.0	150	15	10
3.0	225	22.5	15
4.0	300	30	20
5.0	375	37.5	25

DURATION OF TREATMENT:

Up to 60 months.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, and anti-PEG-PAL IgE) will be assessed.

Safety:

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in a previous rAvPAL-PEG study may be enrolled into this study.
No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseline, of vital signs, ECG results, and X-ray results will also be provided.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

For subjects who have received any study drug in this study with any post-treatment blood Phe concentration measurements and diet diary information, the relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will be explored.

Substudy Analysis:

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

[redacted]

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular

DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury

NAb	neutralizing antibodies
NCI	National Cancer Institute
NOAEL	no observable adverse effect level
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Regulatory Affairs Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to

normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given $\geq$ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience as of JAN2011 from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.

7.2.1 Phase 1 Study PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.

- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002, PAL-004, and 165-205

Currently, rAvPAL-PEG is being investigated in three Phase 2 clinical trials (PAL-002 and PAL-004). As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in a rAvPAL-PEG Phase 2 study.

In PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU), approximately 55 subjects will be enrolled and will be administered multiple doses of rAvPAL-PEG up to 5.0 mg/kg/week. PAL-002 is a 2-part study. In Part 1, subjects will be assigned to 1 of 4 rAvPAL-PEG dose cohorts (0.001, 0.003, 0.01, 0.1 mg/kg, or 1.0 mg/kg). rAvPAL-PEG will be administered at a fixed weekly dose of 0.001 mg/kg (Cohort 1), 0.003 mg/kg (Cohort 2), 0.01 mg/kg (Cohort 3), 1.0 mg/kg (Cohort 4), or 0.1 mg/kg (Part 1 Substudy) for 8 weeks. In Part 2, all subjects who complete Part 1 will be administered an adjustable dose of rAvPAL-PEG for up to an additional 8 weeks. The dose administered in Part 2 may be adjusted by dose level and/or dose frequency to reach a target blood Phe concentration of 60-600 µmol/L.

Study PAL-003 (Long-Term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU) is an extension study of PAL-002, PAL-004 and 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy and Tolerability of Subcutaneously Administered rAvPAL-PEG in Patients with PKU for 24 Weeks). Once subjects have completed a previous rAvPAL-PEG study, they have the option to enroll into PAL-003 and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months.

Study PAL-002 was initiated in September 2009 and is ongoing. The primary objective of this study is to evaluate the effect of multiple doses of rAvPAL-PEG on blood Phe concentrations in subjects with PKU for up to 16 weeks of treatment. The secondary objectives of the study are (1) to evaluate the safety and tolerability of SC injections of multiple dose levels of rAvPAL-PEG, (2) to evaluate the antibody response to rAvPAL-PEG,

and (3) to evaluate the PK profile of rAvPAL-PEG in subjects with PKU. The study protocol was amended (30APR2010) to include a substudy of an additional rAvPAL-PEG dose level (0.1 mg/kg/week) that reduced blood Phe concentrations to clinically significant levels in the Phase 1 study, PAL-001. The protocol was amended again (28MAR2011) to increase the starting dose for Cohort 4 from 0.03 mg/kg/week to 1.0 mg/kg/week. As of JAN2011, 33 subjects have been enrolled into the study and all 10 subjects of the substudy have been dosed with 0.1 mg/kg rAvPAL-PEG. Additional information about the safety reported as of JAN2011 is provided in Section 7.4.2.

Study PAL-004 is designed to determine if daily administration of rAvPAL-PEG at daily dose levels (0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day) is safe and effective in reducing and maintaining consistent blood Phe concentrations in subjects with PKU. It is hypothesized that a higher starting dose and frequency will reduce blood Phe concentrations to near-normal levels in a timely manner and will provide the greatest clinical benefit. Considering the concentration and volume of drug that is administered subcutaneously, the only way to increase the rAvPAL-PEG dose is via daily administration. Therefore, in Study PAL-004, a higher starting dose of 0.4 mg/kg will be administered 5 days a week (referred to as daily), a dosing regimen that has been observed to be tolerated and to reduce blood Phe concentrations to clinically meaningful levels in previously exposed subjects in this study. Following completion of Study PAL-004, subjects have the option to enroll into this study and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months.

Study 165-205 is a Phase 2 study using a subject-driven dose modification schema to assess safety. In addition, fixed dosing at a drug concentration of 15 mg/mL rAvPAL-PEG will be assessed. As of May 2012, enrollment of subjects is underway for this study.

In this study, subjects who completed a previous rAvPAL-PEG study will continue to be administered the same rAvPAL-PEG dose that was administered in the previous study. If the safety, PK, and blood Phe concentration results from the previous study indicate that the appropriate level of PEGylation and dosing regimen differs from that administered in the previous study, the PEGylation and/or dosing regimen administered in this study may change.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally

elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator in consultation with the Sponsor's Medical Officer. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is

little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

This study is an extension of previous rAvPAL-PEG studies. Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from previous rAvPAL-PEG studies will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to PEG

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to an Immunologic Reaction

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo

and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers, sedimentation rates, and complete blood counts (CBCs).

As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in the single-dose, Phase 1 study (PAL-001) and in the repeat -dosing Phase 2 studies (PAL-002, PAL-004, and PAL-003). The dose levels in the repeat-dosing studies range from 0.001 to 5.0 mg/kg/week administered in various frequencies (including 5 days a week). The most clinically significant AEs have been hypersensitivity reactions that have led to dosing interruptions and reductions. Most of the hypersensitivity reactions have been nonserious and mild-to-moderate in severity.

A total of 4 SAEs have been reported in the previous rAvPAL-PEG studies. Of these 4 SAEs, 3 have been reported as related to rAvPAL-PEG. Two of the study-drug related SAEs occurred in Study PAL-001. Both of these SAEs occurred in subjects in the lower dose cohorts: an SAE of hypersensitivity reaction in a subject in the 0.001 mg/kg cohort and an SAE of anaphylactic reaction (urticaria) in a subject in the 0.01 mg/kg cohort. Neither SAE was severe or resulted in discontinuation from the study, and both of the SAEs resolved. There were no SAEs in subjects in the higher dose cohorts (0.03 mg/kg and 0.1mg/kg) in PAL-001. The third study drug-related SAE (angioedema) occurred in a subject in Study PAL-004. The fourth SAE (urticaria, dehydration) was reported as not related to study drug and occurred in a subject in Study PAL-002. There have been no reports of anaphylaxis for any subject treated with rAvPEG-PAL (as of January 2012).

Subjects who have a systemic clinical reaction at any time during this study may undergo a series of assessments to monitor safety, including assessment of complements, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and IgE antibodies. Reintroduction of rAvPAL-PEG dosing after a reaction will depend on clinical assessments and available laboratory data, such as chemistry and urine studies, to ensure that there has been no

end-organ damage prior to resuming treatment with rAvPAL-PEG. As of May 2012, antibody titers, including positive IgE titers, have not been predictive of future clinical reactions when dosing with rAvPAL-PEG. Additionally, subjects will be premedicated to prevent or mitigate risk of hypersensitivity reactions (refer to Section 9.1.1.3.3).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.), however, antibodies against PEG may form when PEG is bound to compounds. (Harris, 2003, Nat.Rev.Drug Discov.),(Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in any significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.). Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. Subjects in the Phase 2 rAvPAL-PEG studies have developed both anti-PEG IgM and anti-PEG IgG antibodies. Antibody formation may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.

7.4.2.1 Systemic Skin Reactions

Two out of 25 subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. One of these events was reported as serious, and the other was reported as nonserious; both events were reported following administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications.

The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects

resolved within 1 day following treatment with medication, including the [redacted]. Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. Upon study completion, the subject continued to receive regular doses (every 3 months) of Depo-Provera, and no further reactions were reported. The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist. See Section 7.2.1 for the results from Study PAL-001.

The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. The results from nonclinical studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys indicated the formation of anti-rAvPAL antibody titers that did not correspond with observations of injection-site reactions. Quantitation of anti-PEG titers were not assessed in the chronic repeat-dose studies in the rat and monkey. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to, during, and after this study is prohibited as a precautionary measure (refer to Section 9.3.2 and Section 9.4.8).

7.4.2.2 Management of Allergic Reactions

Allergic reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30-60 minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of allergic reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the

rAvPAL-PEG injection. Subjects who qualify for self administration of study drug will be provided with emergency resuscitation instructions (refer to Section 9.4 and the Subject Self-Administration Training Materials).

In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of allergic symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oral or IV glucocorticoids.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment (eg, acetaminiophen or ibuprofen).
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. An allergy and/or immunology consultation should be sought if necessary. Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.1.1. Detailed instructions for the management of allergic reactions are provided in the Study Reference Manual.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objective is as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of rAvPAL-PEG Phase 2 studies in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity was observed in a previous rAvPAL-PEG studies.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigator in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to clinic monthly for review with the clinical study staff.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self administration. Additional information is provided in the Subject Self-Administration Training Materials.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose (subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks; refer to Section 9.4.4). Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 9.1.1 and Table 9.1.2. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator.
- The subject has completed the study through the Month 60 visit.
- The study is terminated.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2.

The Schedule of Events and PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2

Table 9.1.1: Schedule of Events

		Treatment Period[b,c]								
		Week 1	Weekly[b]	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)	Final F/U Visit	Self Administra-tion	Early Term. Visit	
Assessments and Events	**Screening[a]**	**D 1**	**Starting at Week 2**	**Every 4th week; Perform in addition to weekly procedures**	**Every 12th week. Perform in addition to monthly procedures**	**Up to daily**	**1 week after final dose**		**4 weeks after final dose**	**Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours**
Informed consent	X									
Medical history, including allergy history, and demographics	X									
Physical examination[e]	X	X			X		X		X	
Vital signs[e]	X	X	X			X			X	X
Weight	X			X						
12-lead ECG	X						X		X	

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours
		Week 1	Weekly[b]	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
Clinical laboratory tests[f]	X			X			X		X	X[g]
Complements C_3 and C_4[h]	X				X					X
Sedimentation rate		X			X		X		X	X
Chest x-ray	X			X (Week 48 visit only)			X		X	
Urine pregnancy test[i]	X	X		X			X		X	
Injection-site inspectionj[i j]		X (postdose)	X			X	X	X	X	X

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours
		Week 1	Weekly[b]	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
Adverse events[j, k]	X	X	X			X	X	X	X	X
Weekly phone call to self admin participants only (to assess AEs, Inj site reactions, concomitant medications)								X	X	
Concomitant medications[j]	X	X	X			X	X	X	X	X
Diet query	X	X	X				X		X	
3-Day diet diary[l]				X			X		X	
Serum antibodies		X		X			X		X	X
Plasma Phe and plasma	X	X	X	X[m]			X		X	

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 48 hours
		Week 1	Weekly[b]	Monthly (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week; Perform in addition to weekly procedures	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
tyrosine[m]										
Plasma PK sample		X	X[o]				X		X	X
Administer study drug[n]		X	X			X		X		
Skin biopsy (optional; affected and not affected area)										X
Serum tryptase level [o]										X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] A separate Screening visit for this study is required only if the time between completion of the previous rAvPAL-PEG study and enrollment into PAL-003 is greater than 28 days.

[b] Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. Monthly visits must be performed in the clinic.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Refer to Section 9.1.1.3.3 and Section 12.3.8.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis. Urine microscopy will be performed if any urinalysis results are positive for hematuria. Refer to Table 9.7.6.1.

[g] Subjects who have a systemic reaction or a large local reaction not contiguous with the injection site should be assessed for CRP, CH50, C_1, C_3, and C_4 within 48 hours of the reaction.

[h]Complement C_3 and C_4 will be collected at the Screening Visit and quarterly. Additional complement testing will be performed and as needed to resolve previous abnormal test results.

[i] If positive or equivocal, perform serum pregnancy test.

[j] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm).

[j] Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

[k] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.

[m] Samples should be drawn at least 2.5 hours after a meal. At the Investigator's discretion, blood Phe may be collected monthly rather than weekly, provided the subject satisfies the criteria in Section 9.7.2.1.

[n] Dosing is up to 5.0 mg/kg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may be re-evaluated.

[o] Mandatory for subjects who have a systemic reaction. Take sample immediately after reaction if possible.

Table 9.1.2: PK Substudy Dosing Regimens

Treatment Frequency	Plasma PK Sampling	Example
Subjects who are administered study drug once per week	Pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours when possible), and pre-dose of the next weekly dose	If dosed on Monday: Obtain PK sample pre-dose when possible – pre-dose Monday, Tuesday, Wednesday, Thursday, Friday, and pre-dose the following Monday. No further PK substudy draws.
Subjects who are administered study drug two or three times per week	Pre-dose and every 24 hours during the longest period between doses, when possible, and pre-dose of the next dose.	If dosed on Monday, Thursday, and Friday: Obtain PK sample every 24 hours during the longest period between doses: Pre-dose Monday, Tuesday, Wednesday, and pre-dose Thursday. No further PK substudy draws.
Subjects who are administered study drug four to seven times per week.	Pre-dose and every 12 hours during the longest period between doses, when possible, then pre-dose of the next dose.	If dosed Monday through Friday: Obtain PK sample every 12 hours during the longest period between doses (Friday to Monday): pre-dose Friday, 12 hours post-dose Friday, Saturday (24 and 36 hours post dose), Sunday (48 and 60 hours post-dose), and pre-dose the following Monday. No further PK substudy draws.

9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG

Subjects who qualify for self administration of study drug will be provided with information and instruction with regard to management of local and systemic reactions (refer to the Subject Self-Administration Training Materials).

9.1.1.1 Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product

Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from a previous rAvPAL-PEG study are excluded from participation in this study. Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in a previous rAvPAL-PEG study are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing for the remainder of the study. The premedication dosage will be standard. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable). Subjects may develop systemic, large local skin, or local skin reactions after enrollment in PAL-003. Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1

9.1.1.2 Definition of Reaction and Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously

During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows:

Local skin reaction:

- Skin signs or symptoms in 1 affected primary location, ie, hives, wheals, or swelling or an area of erythema, redness, induration, pain, or itching at or near the site of injection.

Large local skin reaction:

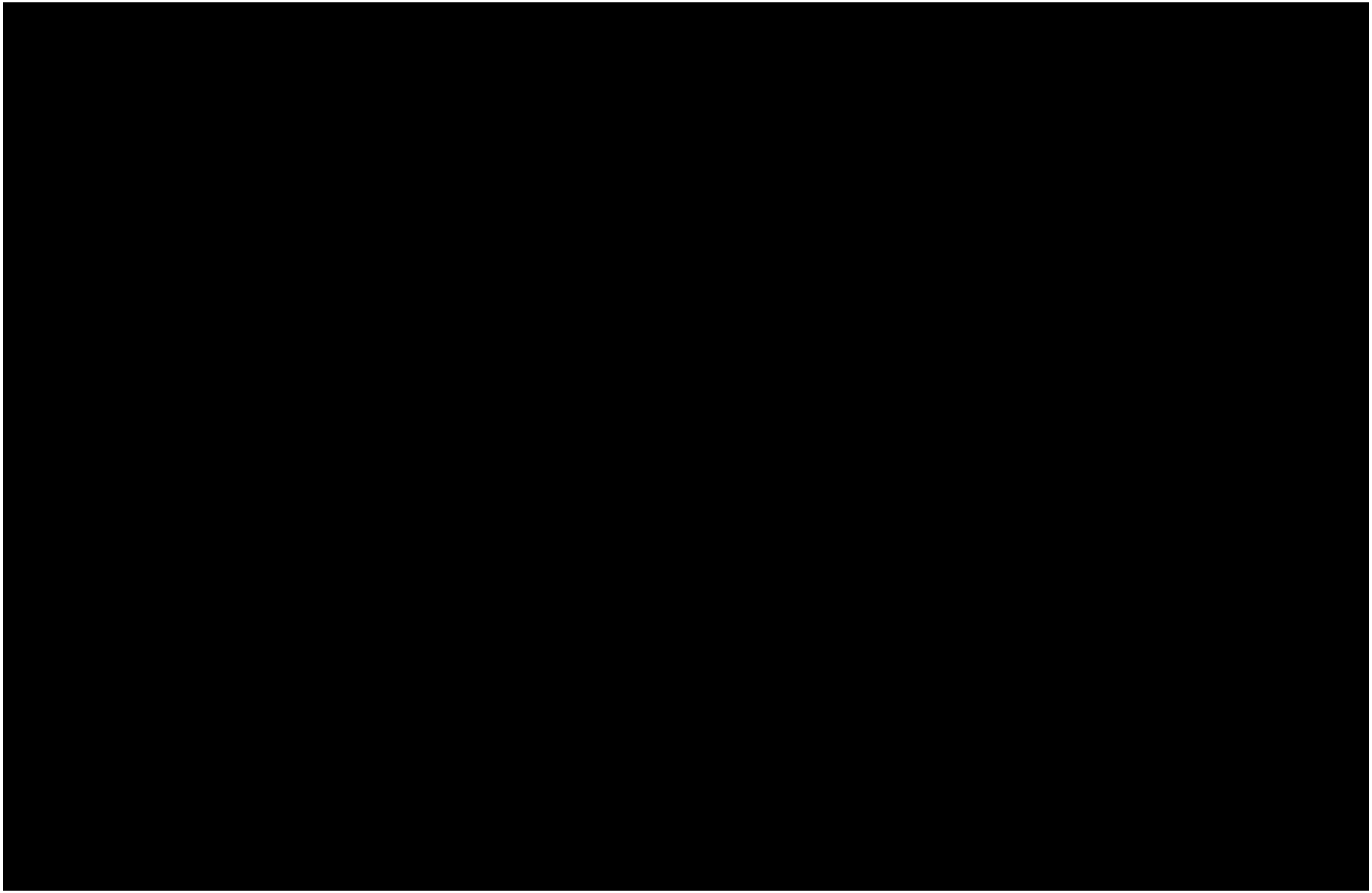
- Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site.

Systemic reaction (including generalized skin symptoms):

- Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological; and/or a fever attributed to treatment with rAvPAL-PEG (≥ 100.4°F or ≥ 38°C).

For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1

[redacted]



9.1.1.3.1 Local Skin Reactions

All subjects who experience a local skin reaction during this study may be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Recommended premedications are acetaminophen and/or non-sedating antihistamine. The dosage will be standard. Because antihistamines can cause drowsiness, sedating antihistamines may be administered only if the subject is accompanied by a designated driver. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2).

If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from study treatment.

9.1.1.3.2 Large Local Skin Reactions

Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects will be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site

Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. For the remainder of the study, subjects will be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site

Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction (refer to Section 9.1.1.3.3). An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.3 Systemic Reactions

Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG must stop further administrations of rAvPAL-PEG and must immediately return to the clinic for safety assessments. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 48 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Reaction Visit): serum antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti rAvPAL-PEG IgE, anti-rAvPAL-PEG neutralizing antibodies); serum tryptase level (it is recommended that this sample be drawn immediately after reaction); sedimentation rate; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1.

Following the reaction, subjects will be required to complete the assessments of the Unscheduled Hypersensitivity Reaction visit, including assessment of IgE. If a subject presents with a clinical diagnosis of anaphylaxis, further dosing will be held while laboratory evaluation of IgE, as part of the Unscheduled Systemic Reaction Visit, is performed. Subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 1 week. If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing

will not resume until review of the case with the Investigator, Sponsor's Medical Officer, and an allergist/immunologist.

rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects must be premedicated orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30-60 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section 9.1.2. Subjects must be premedicated orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30-60 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

9.1.2 Dose Modifications

9.1.2.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator, in agreement with the Sponsor's Medical Officer. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.

- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg) or per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg. Subjects who increase their dose frequency should perform the Interim Dosing Visit assessments (refer to Section 12.3.6 and Table 9.1.1).
- When a dose is increased, the subject must be observed for 30-60 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

9.1.2.2 Dose Decrease Methodology

All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer. Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for safety (ie, any adverse event that may be improved with a lower, more frequent dose).

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required to attain the target Phe concentration, dosing frequency may be reduced.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L as measured by serum Phe levels for a minimum of 1 month), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

9.1.3 Safety Assessment Criteria

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principle Investigator (PI) and the Sponsor's Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

9.1.4 Stopping Criteria

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.

5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from a previous rAvPAL-PEG study.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study treatment should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study treatment include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in their previous rAvPAL-PEG study. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose that was administered upon completion of that study provided that there was no interruption in dosing. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed to by the PI and the Sponsor's Medical Officer, based upon the subject's response to doses.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects may be eligible to self administer study drug if he or she meets the following criteria:

- The subject is on a stable dosing regimen for 2 weeks (ie, the subject has demonstrated a blood Phe level within 60-600 µmol/L for a minimum of 2 consecutive weeks).
- The subject has not experienced any CTCAE Grade 3 or higher adverse event.
- The subject has not experienced any hypersensitivity reaction to rAvPAL-PEG for at least 4 weeks.
- The subject has no cognitive impairments that may increase the safety risk of self administration per the assessment of the PI.
- The subject has been approved for self administration of study drug by the Sponsor's Medical Officer.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with epinephrine and has been trained on when and how to administer it.

Qualified study site personnel will train each eligible subject on all procedures for self administration of study drug under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self administer a dose at home. The subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 9.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials.

Subjects who are eligible for self administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.

- How to safely use and dispose of syringes used for injections of study drug.
- How to use a new syringe and vial every time drug is administered.
- How to care for their injection site after an injection of study drug.
- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine.
- Who to contact at the study site in case of an emergency.

The PI or the Sponsor's Medical Officer may request that self administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplying the study drug to subjects who qualify for self administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg of rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.

Dilution instructions are provided in a separate instruction manual.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

IP must be stored at 5 ±3° C (41 ± 5° F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the Sponsor's Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose).

For subjects receiving weight-based dosing, dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive weeks) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1.

Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 10 mg/ml concentration (ml)	Vol for 15 mg/ml concentration (ml)
0.03	2.5	0.25	0.17
0.06	5	0.5	0.33
0.12	10	1	0.67
0.25	20	2	1.33
0.5	40	4	2.67
1.0	75	7.5	5
2.0	150	15	10
3.0	225	22.5	15
4.0	300	30	20
5.0	375	37.5	25

It is preferable that the injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.2 as an example. Note this table is for example purposes only. Dosage

calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff, other qualified and trained study personnel, or qualified subjects.

Information for dosing of subjects who enroll into this study after completing previous rAvPAL studies is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

Table 9.4.4.2: Number of Injections Required For an Individual Weighing 80 kg

Dose Group (mg/kg)	Weight (kg)	Volume of Study Drug (mL)[a]	No. of Injections[b]
0.001	80	0.8	1
0.003	80	0.6	1
0.01	80	0.8	1
0.03	80	0.2	1
0.06	80	0.5	1
0.1	80	0.8	1
0.3	80	2.4	2
0.6	80	4.8	2 or 3
1.0	80	8.0	4

aStudy drug is supplied at a concentration of 10 mg/mL and is then diluted by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume.

[b]Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.

Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may need to be re-evaluated (refer to Section 9.4.10).

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in their previous rAvPAL-PEG study.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the Sponsor's Medical Officer must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and/or non-sedating antihistamines (refer to Section 9.1.1). If the local skin reaction worsens with a repeat

injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior systemic reaction to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.1.1).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for dosing of subjects who enroll into this study after completing previous rAvPAL_PEG studies is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator in consultation with the Sponsor's Medical Officer. Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1 during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study treatment per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific

accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigator in consultation with the Sponsor's Medical Officer.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next monthly study visit.The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a]	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Pregnancy test (urine) and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1
Urinalysis	Central laboratory	9.7.6

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1).

Blood Phe may be collected monthly, rather than weekly, at the discretion of the Investigator and provided that the subject meets the following criteria:

- Is receiving a stable dose of rAvPAL-PEG (defined as no dose modifications or interruptions for at least the previous 2 weeks)
- Has a stable blood Phe level (defined as Phe between 60-600 µmol/L for at least 2 consecutive weeks)

The subject may continue to have monthly, rather than weekly, blood Phe collection provided the following do not occur:

- Blood Phe measurement > 600 µmol/L
- Blood Phe measurement ≤ 30 µmol/L

If either of these conditions occurs, then that subject should return to weekly blood Phe draws until the rAvPAL-PEG dose has been adjusted and the Phe level is again within the protocol-defined target range for at least 2 weeks.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed. For subjects participating in the Substudy, PK sampling will be performed as follows:

- For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.
- For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose.
- For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

See Table 9.1.2 for additional details.

BioMarin will perform the analysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PAL IgE, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1.3.3). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1).

BioMarin will perform all anti-body testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Specific days for obtaining samples are provided in Table 9.1.1 and in Section 12.
The scheduled clinical laboratory tests are listed in Table 9.7.6.1. A central laboratory will be used for analysis.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Phenylalanine
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Sedimentation rate[a]
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Spot urine albumin/creatinine ratio	**Additional Unscheduled Hypersensitivity Reaction Visit Tests[b]**
Total protein		Nitrite	CH50
Calcium		Urobilinogen	C_1, C_3, C_4
Sodium		Hemoglobin	Serum tryptase level[b]
Potassium		Bilirubin	CRP
Glucose			
Uric acid			**Complement Testing[c]**
CO_2			C_3
Chloride			C_4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory.

[b] Perform immediately after reaction if possible.

[c] Complement C_3 and C_4 to be drawn at the Screening Visit and then quarterly or as needed to resolve abnormal test results.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study period during which all non-serious AEs and SAEs will be reported begins after informed consent is obtained and the first administration of study drug and continues until 4 weeks following the last administration of study drug or the last visit of the treatment period (refer to Section 12). After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. Resolution of AEs (with dates) should be documented on the appropriate CRF page(s) and in the subject’s medical record.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated

with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)
4	Life threatening or debilitating: consequences; urgent intervention indicated[b]
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	**Description**
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
    - Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Is an important medical event or reaction.

The reporting period for SAEs begins after informed consent is obtained, and continues until 4 weeks following the last administration of study drug or End of Treatment Visit.

All SAEs, whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form to BioMarin Pharmacovigilance (BPV).). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the subject's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution on the Pregnancy Follow-up Form in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the

IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (i.e., modified dose amount or frequency not defined in protocol).
- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.5 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the Sponsor's Medical Officer any AEs for which the issue of seriousness is unclear or questioned. Contact information for the Sponsor's Medical Officer is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to clinic on a monthly basis for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis)
- Complement C_3 and C_4
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week

unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Sponsor's Medical Officer)

12.3.2 Weekly Visits

Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. However, dose modifications must be performed in the clinic. Subjects must have AEs and

concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. For subjects on a stable dose of rAvPAL-PEG (ie, no dose change in the last 2 weeks) and have had a phenylalanine (Phe) level at or below target range (60-600 µmol/L) for 2 consecutive weeks weekly visits may be waived and replaced by a telephone call per discretion of the Investigator but monthly visits are required.

The following study activities will be performed at the weekly visits beginning with Week 2:

- Vital signs
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
    - Blood Phe may be collected monthly, rather than weekly, if certain criteria are satisfied (refer to Section 9.7.2.1).
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Sponsor's Medical Officer)
- Weekly telephone call
    - For those subjects performing self administration and waiving (per Investigator discretion) the weekly visit (refer to Section 12.3.7).

12.3.3 Substudy Visits

For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2.

12.3.4 Monthly Visits (Week 4, 8, 12, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 4, monthly visits must be performed in the clinic and consist of all weekly activities listed above in Section 12.3.2 as well as the additional study activities listed below:

- Weight
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray.
- Chest x-ray (Week 48 visit only)
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.)
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Weekly telephone call
    - For those subjects performing self administration and waiving (per Investigator discretion) the weekly visit (refer to Section 12.3.7).

12.3.5 Quarterly Visits (Week 12, 24, 36, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 12, quarterly visits consist of all weekly (Section 12.3.2) and monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below:

- Physical examination
- Complement C_3 and C_4
- Sedimentation rate

12.3.6 Interim Dosing Visit

Subjects who increase their dose frequency during the study from 1x/week to 2x/week or daily will have additional assessments performed for 5 weeks after the subject's rAvPAL-PEG dose frequency has been increased. If the Interim Dosing Visit coincides with a weekly, monthly, or quarterly scheduled visit, the scheduled visit assessments should also be performed. The following study activities will be performed for the Interim Dosing Visit:

- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Administer study drug

12.3.7 Weekly Telephone Visit

Subjects who are administered rAvPAL-PEG at home and/or have been approved to self administered rAvPAL-PEG, should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:

- Injection-site self inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Confirmation of drug administration

12.3.8 Unscheduled Hypersensitivity Reaction Visit

Subjects who have a systemic reaction, including a generalized skin reaction, or a large local skin reaction that is not contiguous to the injection site after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 48 hours) the reaction.

- Injection-site inspection
- Clinical laboratory tests
- Sedimentation rate
- CRP, CH50, C1, C3, and C4
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies)
- Serum tryptase level
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction)
- PK sample
- Assessment of AEs

- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 8 weeks. If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing will not resume until review of the case with the Investigator, Sponsor's Medical Officer and an allergist/immunologist (refer to Section 9.1.1.3.3).

12.4 Early Termination Visit

The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who terminate from study treatment early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query

- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

12.5 Final Follow-up Visit

The final follow-up (F/U) Visit will occur 1 week after the final dose of study drug.

The following study activities will be performed at the F/U Visit:

- Physical examination
- Vital signs, including weight
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Final Follow-up Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics, including clinically significant changes from baseline, of vital signs, ECG results, and X-ray results will also be provided.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should data become available from previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

14.3.1 Pharmacokinetic Substudy Analysis

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis. Information regarding this exploratory analysis will be provided in the Statistical Analysis Plan.

For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored. Information regarding the analysis will be provided in the Statistical Analysis Plan.

14.5 Determination of Sample Size

Subjects who participated in previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study. Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Sponsor's Medical Officer. The Chair will be notified by the Sponsor's Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study, on a schedule defined in the DMC Charter, and offer advice on whether or not to proceed, modify or terminate study enrollment on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.4.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment. If the subject has followed the Investigator's instructions, BioMarin will pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures, if these costs are not covered by health insurance or another third party that usually pays these costs. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records.
In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then

set the status of the forms, visits, and the entire casebook to locked. Upon completion of the clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

[redacted]

June 11, 2012

[redacted]

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (see pages 2-4). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough font~~.

Section No./Title	Revision	Rationale
Section 2/Synopsis, Study Rationale	… This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205~~the dose-finding studies (Study PAL-002 and Study PAL-004).~~ Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects. ~~Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments.~~ A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.	5, 6, 16

Section 2/Synopsis, Objectives	The exploratory objective of the study is as follows: • To ~~assess~~ evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU. The Substudy objectives are as follows: • To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned. • ~~To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU.~~ • ~~To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU.~~ • ~~To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU.~~ • ~~To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.~~	5, 6, 16

Section 2/Synopsis, Study Design and Plan	This is a long-term extension of rAvPAL-PEG Phase 2 studies~~the Phase 2, open-label, dose-finding studies, PAL-002 and PAL-004,~~ in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity is observed in a ~~PAL-002 or PAL-004~~previous rAvPAL-PEG study~~004~~. Only subjects who completed ~~the protocol-defined study drug regimen in PAL-002 or PAL-004~~a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. ~~Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004.~~ The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration~~due to restarting dosing in this study~~. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the ~~Investigaor~~Investigator in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. ~~PAL-002 and PAL-004~~ Subjects' previous ~~subjects'~~ rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose (subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks). Doses will be evaluated on an individual basis.	4, 6

Section 2/Synopsis, Study Design and Plan	PAL-003 Substudy A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed.~~A subset of up to 10 subjects who are already enrolled and who have consented to participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption. To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.~~	5, 6
Section 2/Synopsis, Dose Increase Methodology	~~For subjects from PAL-002 only,~~ A~~a~~n individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows. • Only 1 dose ~~(level or frequency)~~ adjustment is allowed every 2 weeks. • ~~Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.~~	2, 7

Section 2/Synopsis, Dose Decrease Methodology	If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L as measured by serum Phe levels for a minimum of 1 month), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).	8
Section 2/Synopsis, Diagnosis and All Criteria for Inclusion and Exclusion	Individuals eligible to participate in this study must meet all of the following criteria: 1. Must have completed participation ~~and all protocol-defined study drug in PAL-002 or PAL-004~~in a previous rAvPAL-PEG study. Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study: 4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction ~~of generalized rash~~ may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study. 8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from ~~PAL-002 or PAL-~~ a ~~004~~previous rAvPAL-PEG study~~004~~.	11

Section 2/Synopsis, Investigational Product, Dose, Route, and Regimen	rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg or rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product. Upon enrollment into this study, subjects from previous rAvPAL-PEG studies ~~PAL-002~~ will be dosed with the same or higher dose than the dose that was administered upon completion of that study provided that there was no interruption in dosing. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer ~~PAL-002. Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004.~~ rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination in consultation with the Sponsor's Medical Officer based on ~~on~~available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 6~~12~~0-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the ~~Study~~ Sponsor's Medical Officer, based upon the subject's response to doses. Fixed Dosing Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive weeks) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects should convert their weight-based dose using the following table:	4, 16

<table>
<tr><td>Section 2/Synopsis, Investigational Product, Dose, Route, and Regimen</td><td><table>
<tr><th>Weight-based dose (mg/kg)</th><th>Equivalent fixed dose (mg)</th><th>Vol for 10 mg/ml concentration (ml)</th><th>Vol for 15 mg/ml concentration (ml)</th></tr>
<tr><td>0.03</td><td>2.5</td><td>0.25</td><td>0.17</td></tr>
<tr><td>0.06</td><td>5</td><td>0.5</td><td>0.33</td></tr>
<tr><td>0.12</td><td>10</td><td>1</td><td>0.67</td></tr>
<tr><td>0.25</td><td>20</td><td>2</td><td>1.33</td></tr>
<tr><td>0.5</td><td>40</td><td>4</td><td>2.67</td></tr>
<tr><td>1.0</td><td>75</td><td>7.5</td><td>5</td></tr>
<tr><td>2.0</td><td>150</td><td>15</td><td>10</td></tr>
<tr><td>3.0</td><td>225</td><td>22.5</td><td>15</td></tr>
<tr><td>4.0</td><td>300</td><td>30</td><td>20</td></tr>
<tr><td>5.0</td><td>375</td><td>37.5</td><td>25</td></tr>
</table></td><td>4</td></tr>
<tr><td>Section 2/Synopsis, Sample Size</td><td>Subjects who participated in ~~PAL-002 or PAL-004~~a previous rAvPAL-PEG study may be enrolled into this study.
No formal sample size calculation was conducted for this study or the PAL-003 Substudy.</td><td>16</td></tr>
<tr><td>Section 2/Synopsis, Substudy Analysis</td><td>For subjects who completed the Substudy, the ~~effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response~~steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.</td><td>5, 6</td></tr>
<tr><td>Section 6/Investigators and Study Administrative Structure</td><td>Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR)~~, [urinalysis]~~, and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.</td><td>12</td></tr>
</table>

Section 7.2/Previous Clinical Studies	The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002,~~and~~Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience ~~to date~~as of ~~(~~JAN2011~~)~~ from this ongoing study and the other ongoing Phase 2 studies~~y~~, PAL-002 and PAL-004.	16
Section 7.2.2/Phase 2 Studies PAL-002, PAL-004, and 165-205	Currently, rAvPAL-PEG is being investigated in ~~two~~ three Phase 2 clinical trials (PAL-002 and PAL-004). ~~To date (JAN2011)~~As of May 2012, a total of 7033 subjects have been administered rAvPAL-PEG in ~~Study PAL-002~~a rAvPAL-PEG Phase 2 study~~and initiation and recruitment of clinical sites is underway for Study PAL-004~~. Study PAL-003 (Long-Term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU) is an extension study of PAL-002,~~and~~ PAL-004, and 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy and Tolerability of Subcutaneously Administered rAvPAL-PEG in Patients with PKU for 24 Weeks). Once subjects have completed ~~Study PAL-002 or PAL-~~a p~~004~~revious rAvPAL-PEG study~~004~~, they have the option to enroll into PAL-003 and to continue to receive doses of rAvPAL-PEG for up to an additional 60 months. …. The protocol was amended again (28MAR2011) to increase the starting dose for Cohort 4 from 0.03 mg/kg/week to 1.0 mg/kg/week. ~~To date (~~As of JAN2011~~)~~, 33 subjects have been enrolled into the study and all 10 subjects of the substudy have been dosed with 0.1 mg/kg rAvPAL-PEG. Additional information about the safety reported as of ~~to date (~~JAN2011~~)~~ is provided in Section 7.4.2. Study 165-205 is a Phase 2 study using a subject-driven dose modification schema to assess safety. In addition, fixed dosing at a drug concentration of 15 mg/mL rAvPAL-PEG will be assessed. As of May 2012, enrollment of subjects is underway for this study. In this study, subjects ~~in~~who completed a previous rAvPAL-PEG study ~~PAL-002 and PAL-004~~ will continue to be administered the same rAvPAL-PEG dose that was administered in the previous study~~PAL-002 or PAL-004~~. If the safety, PK, and blood Phe concentration results from the previous study~~either PAL-002 or PAL-004~~ indicate that the appropriate level of PEGylation and dosing regimen differs from that administered in the previous study~~PAL-002 or PAL-004~~, the PEGylation and/or dosing regimen administered in this study may change.	15

Section 7.3/Study Rationale	~~Because rAvPAL-PEG is being assessed as a long-term chronic treatment for patients with PKU, there is a need to understand the effect of a temporary dosing interruption on the safety, efficacy (blood Phe), PK, and immune response. To date (JAN2011), there is limited information on the effects of a rAvPAL-PEG dose interruption in subjects with PKU. Therefore, a Substudy has been added to this study to assess the effects of stopping and re-starting rAvPAL-PEG treatment in subjects who are on a variety of rAvPAL-PEG dosing regimens (dose level and frequency). Up to 10 subjects who are on a variety of dosing regimens (dose level and frequency) in this study are eligible for participation in the Substudy and will stop dosing for a period of approximately 4 weeks. During this dosing interruption, subjects will be assessed for safety, efficacy, PK, and immune response and will continue to perform the scheduled study assessments. There is no information regarding the pharmacokinetic (PK) profile of rAvPAL-PEG when blood Phe levels are at 60-600 µmol/L for subjects on a multiple-dose regimen. To address this lack of information, additional PK sampling will be performed prior to the dosing interruption. Upon restarting rAvPAL-PEG, PK sampling will also be performed to allow for collection of drug exposure data (area under the plasma concentration-time curve [AUC] and maximum plasma concentration [C_{max}]), absorption rate, and clearance for subjects who have been previously exposed to rAvPAL-PEG in this Substudy. This information will allow for comparisons to be performed with subjects who were naïve to previous rAvPAL-PEG treatment from the Phase 1 study, PAL-001.~~	6, 15
Section 7.3/Study Rationale	A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range. This study is an extension of previous rAvPAL-PEG~~the the dose-finding~~ studies~~(PAL-002 and PAL-004)~~. Administration of rAvPAL-PEG will be continued to assess whether long-term dosing of rAvPAL-PEG is safe and can maintain reduced blood Phe concentrations in PKU subjects.	5
Section 7.4/Summary of Overall Risks and Benefits	It is not expected that data from previous rAvPAL-PEG studies ~~PAL-002 or PAL-004~~ will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use….	16

Section 7.4.2/Toxicity Due to an Immunologic Reaction	As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in the single-dose, Phase 1 study (PAL-001) and in the repeat -dosing Phase 2 studies (PAL-002, PAL-004, and PAL-003). The dose levels in the repeat-dosing studies range from 0.001 to 5.0 mg/kg/week administered in various frequencies (including 5 days a week). The most clinically significant AEs have been hypersensitivity reactions that have led to dosing interruptions and reductions. Most of the hypersensitivity reactions have been nonserious and mild-to-moderate in severity. A total of 4 SAEs have been reported in the previous rAvPAL-PEG studies. Of these 4 SAEs, 3 have been reported as related to rAvPAL-PEG. Two of the study-drug related SAEs occurred in Study PAL-001. Both of these SAEs occurred in subjects in the lower dose cohorts: an SAE of hypersensitivity reaction in a subject in the 0.001 mg/kg cohort and an SAE of anaphylactic reaction (urticaria) in a subject in the 0.01 mg/kg cohort. Neither SAE was severe or resulted in discontinuation from the study, and both of the SAEs resolved. There were no SAEs in subjects in the higher dose cohorts (0.03 mg/kg and 0.1mg/kg) in PAL-001. The third study drug-related SAE (angioedema) occurred in a subject in Study PAL-004. The fourth SAE (urticaria, dehydration) was reported as not related to study drug and occurred in a subject in Study PAL-002. There have been no reports of anaphylaxis for any subject treated with rAvPEG-PAL (as of January 2012).	15
Section 7.4.2/Toxicity Due to an Immunologic Reaction	Subjects who have a systemic clinical reaction at any time during this study may undergo a series of assessments to monitor safety, including assessment of complements, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and IgE antibodies. Reintroduction of rAvPAL-PEG dosing after a reaction will depend on clinical assessments and available laboratory data, such as chemistry and urine studies, to ensure that there has been no end-organ damage prior to resuming treatment with rAvPAL-PEG. As of May 2012, antibody titers, including positive IgE titers, have not been predictive of future clinical reactions when dosing with rAvPAL-PEG. Additionally, subjects will be premedicated to prevent or mitigate risk of hypersensitivity reactions (refer to Section 9.1.1.3.3). ~~In Study PAL-001, there were 11 subjects (out of 25 subjects in the study) with skin-related reactions (injection-site bruising, erythema, pain, rash, swelling, and urticaria) that occurred following the single administration of rAvPAL-PEG. For all of these subjects, the reactions were generalized skin reactions or injection-site skin reactions that did not compromise other organs and were not life-threatening. These suspected antibody-mediated responses were nonserious and mild or moderate in severity. These subjects are not considered to be at significant risk for antibody-mediated reactions with injection of rAvPAL-PEG during PAL-002, PAL-004, or this study; however, specific precautions for these subjects will be taken during this study to monitor subject safety (refer to Section 9.1.1).~~ ~~In Study PAL-002, 33 subjects have been dosed to date (JAN2011), including at dose levels of 2.0 mg/kg/week (or 0.4 mg/kg/5 days a week). Most reactions reported in these subjects have been nonserious and mild or moderate in severity. There has been 1 SAE reported to date; the event was reported as not related to treatment with study drug. There have been no reports of anaphylaxis to date (JAN2011). Subjects who have a systemic reaction during this study will undergo a series of assessments to monitor safety, including assessment of IgE antibodies,~~	1, 15

Section 7.4.2/Toxicity Due to an Immunologic Reaction	~~, prior to determining if dosing may resume. Additionally, subjects who have systemic reactions will be premedicated and will be monitored closely for safety (refer to Section 9.1.1.3.3 for additional information regarding continued treatment of subjects with systemic reactions during the study).~~ …. In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.). Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. Subjects in the Phase 2 rAvPAL-PEG studies have developed both anti-PEG IgM and anti-PEG IgG antibodies. Antibody formation~~This~~ may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.	15
Section 7.4.2.2/Management of Allergic Reactions	In the event of an allergic reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for ~~antibody testing~~immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit. The following measures are recommended for the treatment of allergic symptoms: • Administration of oral or IV glucocorticoids • Administration of oxygen and IV fluids. • Administration of additional symptomatic treatment (eg, acetaminiophen or ibuprofen). In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. ~~Acetaminophen or ibuprofen (5-10 mg/kg) may also be administered.~~ An allergy and/or immunology consultation should be sought if necessary. Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.1.1. Detailed instructions for the management of allergic reactions are provided in the Study Reference Manual.	1, 10, 16
Section 7.4.3/Effects of Stopping and Re-Starting rAvPAL-PEG Treatment	This section has been removed.	6

Section 8/Study Objectives	The exploratory objective of the study is as follows: • To ~~assess~~ evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU. The Substudy objectives are as follows: • To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned. • ~~To assess the safety and tolerability of stopping and re-starting rAvPAL-PEG dosing in a subset of subjects with PKU.~~ • ~~To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on blood Phe concentration in a subset of subjects with PKU.~~ • ~~To evaluate the effect of stopping and re-starting rAvPAL-PEG dosing on immune response in a subset of subjects with PKU.~~ • ~~To assess the effect of multiple doses of rAvPAL-PEG on the PK profile, in particular clearance of drug, when drug is stopped in a subset of subjects with PKU who have been previously exposed to rAvPAL-PEG.~~	5, 6

Section 9.1/Overall Study Design and Plan	This is a long-term extension of rAvPAL-PEG Phase 2 studies~~the Phase 2, open-label, dose-finding studies, PAL-002 and PAL-004,~~ in approximately 100 subjects with PKU. The doses are planned to be in the same range as those tested in PAL-002 and PAL-004 (starting at 0.001 through 5.0 mg/kg/week), provided no dose-limiting toxicity is observed in a ~~PAL-002 or PAL-004~~previous rAvPAL-PEG study~~004~~. Only subjects who completed ~~the protocol-defined study drug regimen in PAL-002 or PAL-004~~a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. ~~Subjects who enroll from PAL-002 will either continue the dose regimen that was administered upon completion of PAL-002 or start dosing at a higher dose per the dose increase instructions. Subjects who enroll from PAL-002 may start this study at a higher dose level provided there was no interruption in dosing since completion of PAL-002 and enrollment into this study. Subjects who enroll from PAL-004 will re-start rAvPAL-PEG dosing in this study at the same dose level and frequency as was administered upon completion of PAL-004.~~ The first dose will be administered in the clinic, and the subject must be observed for 30-60 minutes following administration~~due to restarting dosing in this study~~. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator in consultation with the Sponsor's Medical Officer and must be performed under the supervision of the Investigator in consultation with the Sponsor's Medical Officer. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to ~~each~~ clinic monthly~~visit~~ for review with the clinical study staff. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose (subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks; refer to Section 9.4.4). Doses will be evaluated on an individual basis.	6, 14, 16

Section 9.1/Overall Study Design and Plan	PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. ~~PAL-002 and PAL-004 s~~Subjects'previous rAvPAL-PEG dosing will continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose (subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks; refer to Section 9.4.4). Doses will be evaluated on an individual basis. A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in a minimum of 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive weeks and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2). ~~A subset of up to 10 subjects who are already enrolled and who have consented to participate in this study will be asked to participate in a Substudy to assess the effect that stopping and re-starting treatment with rAvPAL-PEG has on safety, efficacy, PK, and immune response. Subjects will be eligible for participation in the Substudy provided they also meet all of the PAL-003 eligibility criteria, have been on a stable dosing regimen for approximately 4 weeks during this study, have not had any serious adverse events reported during PAL-003, and were not enrolled in Study PAL-004. Subjects participating in the Substudy will have rAvPAL-PEG administration halted for approximately 4 weeks regardless of their dosing regimen (dose level and frequency). rAvPAL-PEG will not be administered during the dose interruption; however, subjects will continue to perform assessments for safety, blood Phe concentration, PK, and immune response. Subjects enrolled into the Substudy will also have additional PK sampling performed prior to the dosing interruption and during the dosing interruption.~~	4, 5
Section 9.1/Overall Study Design and Plan	~~To allow for PK sampling once dosing is stopped, subjects may be admitted to a research facility within 8 hours of stopping dosing where they will reside for at least 24 hours. After the dosing interruption, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. Upon re-starting rAvPAL-PEG, the first dose must be administered in the clinic and the subject must be observed for 30-60 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Additional PK sampling will also be performed for the first week after the dosing interruption.~~ The Schedule of Events and ~~interim dosing~~PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2: ~~, Table 9.1.3 and Table 9.1.4~~.	4, 5
Table 9.1.1/Schedule of Events	This table has been updated to reflect changes in this amendment.	16

Table 9.1.2/PK Substudy Dosing Regimens	This table has been added for the PK Substudy. The previous Table 9.1.2 regarding interim dosing in subjects w4rho increased dose to 2x/daily has been removed.	5, 6
Table 9.1.3/Interim Dosing Visit Schedule, Subjects Whose Dose is Increased to 2x/Week	This table has been removed.	16
Section 9.1.1.1/Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product	Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from a previous ~~PAL-002 or PAL-004~~ rAvPAL-PEG study are excluded from participation in this study. Subjects who experienced a generalized skin rash are included in this category. Subjects who have had a previous local skin reaction to rAvPAL-PEG in ~~PAL-002 or PAL-0~~a~~04~~ previous rAvPAL-PEG study are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or non-sedating antihistamines ~~1 hour~~1-2 hours prior to study drug dosing for the remainder of the study. The premedication dosage will be standard….	3
Section 9.1.1.3.1/Local Skin Reactions	All subjects who experience a local skin reaction during this study may be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Recommended premedications are acetaminophen and/or non-sedating antihistamine. The dosage will be standard. Because antihistamines can cause drowsiness, sedating antihistamines may be administered only if the subject is accompanied by a designated driver. ~~Subjects who have had a local skin reaction (refer to Section 9.1.1.2 for definition) after administration of rAvPAL-PEG in this study may continue if the symptoms do not worsen and no other symptoms have developed but may be premedicated with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug dosing. Because antihistamines can cause drowsiness, it should be administered only if the subject is accompanied by a designated driver.~~	3
Section 9.1.1.3.2/Large Local Skin Reactions	Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects will be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses.~~may be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing.~~ Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).	3

Section 9.1.1.3.2.1/Large Local Skin Reactions Contiguous to Injection Site	…. For the remainder of the study, subjects will be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study dosesmay be premedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to the next dose of study drug. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).	3
Section 9.1.1.3.3/Systemic Reactions	Following the reaction, subjects will be required to complete the assessments of the Unscheduled Hypersensitivity Reaction visit, including assessment of IgE. If a subject presents with a clinical diagnosis of anaphylaxis, further dosing will be held while laboratory evaluation of IgE, as part of the Unscheduled Systemic Reaction Visit, is performed. Subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 1 week. If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing will not resume until review of the case with the Investigator, Sponsor Medical Officer, and an allergist/immunologist. Following completion of the Unscheduled Systemic Reaction Visit, administration of rAvPAL-PEG may resume up to 1 week following the onset of the reaction if the systemic reaction is not IgE-mediated and the subject's safety will not be further compromised with resumed dosing. rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects must be premedicated orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study dosespremedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).	3

Section 9.1.1.3.3/Systemic Reactions	If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section 9.1.2. Subjects must be premedicated orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study dosespremedicated orally with acetaminophen and/or antihistamines (standard dosage per the package insert) 1 hour prior to study drug dosing for the remainder of the study. Additionally, subjects must be closely monitored for 30-60 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).	3
Section 9.1.2.1/Dose Increase Methodology	For subjects from PAL-002An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows: Only 1 dose (level or frequency) adjustment is allowed every 2 weeks. Blood Phe levels will be measured daily for 3 days after each dose increase (fingerstick is acceptable); more frequent or daily blood Phe measurements may be performed.	2, 7
Section 9.1.2.2/Dose Decrease Methodology	If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L as measured by serum Phe levels for a minimum of 1 month), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).	8
Section 9.3.1/Inclusion Criteria	1. Must have completed participation and all protocol-defined study drug in PAL-002 or PAL-004in a previous rAvPAL-PEG study.	11

Section 9.3.2/Exclusion Criteria	4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction ~~of generalized rash~~ may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study. 8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from a ~~PAL-002 or PAL-~~previous rAvPAL-PEG study~~004~~.	16
Section 9.3.4/Subject Identification and Replacement of Subjects	Subjects will retain the same subject number assigned in their previous~~PAL-002 or PAL-~~rAvPAL-PEG study.~~004.~~ This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.	16
Section 9.4/Treatment	Upon enrollment into this study, subjects from previous rAvPAL-PEG studies ~~PAL-002~~ will be dosed with the same or higher dose that was administered upon completion of ~~PAL-002~~that study provided that there was no interruption in dosing. ~~Subjects enrolling from PAL-004, will be administered the same dose that was administered upon completion of PAL-004.~~ rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration…. Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects may be eligible to self administer study drug if he or she meets the following criteria: • The subject is on a stable dosing regimen for 2 weeks (ie, the subject has demonstrated a blood Phe level within 60-600 µmol/L for a minimum of 2 consecutive weeks). …. The ~~patient~~subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess of adverse events, and to answer questions throughout the duration of the study…. Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).	4, 6, 16

Section 9.4.2.1/Product Characteristics and Labeling	The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type-1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg of rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.	4
Section 9.4.4/Directions for Administration	Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, as agreed by the PI and the ~~Study~~ Sponsor's Medical Officer, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose). For subjects receiving weight-based dosing, dosage~~Dosage~~ is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage. Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive weeks) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1. Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen	4

<table>
<tr>
<td>Table 9.4.4.1/ Directions for Administration</td>
<td>
<table>
<tr><th><u>Weight-based dose (mg/kg)</u></th><th><u>Equivalent fixed dose (mg)</u></th><th><u>Vol for 10 mg/ml concentration (ml)</u></th><th><u>Vol for 15 mg/ml concentration (ml)</u></th></tr>
<tr><td><u>0.03</u></td><td><u>2.5</u></td><td><u>0.25</u></td><td><u>0.17</u></td></tr>
<tr><td><u>0.06</u></td><td><u>5</u></td><td><u>0.5</u></td><td><u>0.33</u></td></tr>
<tr><td><u>0.12</u></td><td><u>10</u></td><td><u>1</u></td><td><u>0.67</u></td></tr>
<tr><td><u>0.25</u></td><td><u>20</u></td><td><u>2</u></td><td><u>1.33</u></td></tr>
<tr><td><u>0.5</u></td><td><u>40</u></td><td><u>4</u></td><td><u>2.67</u></td></tr>
<tr><td><u>1.0</u></td><td><u>75</u></td><td><u>7.5</u></td><td><u>5</u></td></tr>
<tr><td><u>2.0</u></td><td><u>150</u></td><td><u>15</u></td><td><u>10</u></td></tr>
<tr><td><u>3.0</u></td><td><u>225</u></td><td><u>22.5</u></td><td><u>15</u></td></tr>
<tr><td><u>4.0</u></td><td><u>300</u></td><td><u>30</u></td><td><u>20</u></td></tr>
<tr><td><u>5.0</u></td><td><u>375</u></td><td><u>37.5</u></td><td><u>25</u></td></tr>
</table>
<u>It is preferable that the</u>~~The~~ injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.
</td>
<td>4, 16</td>
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<tr>
<td>Table 9.4.4.1/ Directions for Administration</td>
<td>…. Study drug will be administered by clinic staff<u>,</u> ~~or~~ other qualified and trained study personnel<u>, or qualified subjects</u>. ~~Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1.~~ Information for dosing of subjects who enroll into this study after completing ~~Study PAL-~~ <u>previous rAvPAL studies</u>~~004~~ is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.</td>
<td>16</td>
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<tr>
<td>Section 9.4.5/Method of Assigning Subjects to Treatment Groups</td>
<td>This will be an open-label study; no randomization schedule will be generated.
Subjects will retain the same subject number used in ~~PAL-002 or PAL-0~~ <u>their previous</u> ~~04~~ <u>rAvPAL-PEG study.</u></td>
<td>16</td>
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Section 9.4.6/Selection of Doses Used in the Study	This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.~~and the ongoing study, PAL-002, to date (JAN2011).~~	16
Section 9.4.8/Prior and Concomitant Medications	Use of any investigational product (with the exception of rAvPAL-PEG used in ~~PAL-002 or PAL-004~~a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be premedicated with acetaminophen and/or non-sedating antihistamines (refer to Section 9.1.1)….	16
Section 9.4.10/Dose Interruption and Missed Doses	~~Information for subjects who are participating in the Substudy and re-start rAvPAL-PEG dosing is provided in Section 9.1.~~ Information for dosing of subjects who enroll into this study after completing ~~Study PAL-002 or Study PAL~~previous rAvPAL PEG studies~~004~~ is provided in Section 9.1.	6, 16
Section 9.6/Dietary or Other Protocol Restrictions	A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next monthly ~~dose of study drug~~ study visit. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.	14
Table 9.7.1.1/Summary of Laboratory Assessments	This table has been updated to reflect changes made to this protocol amendment.	16
Section 9.7.1.3/Vital Sign Measurements	Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, ~~weight (kg),~~ and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.	13
Section 9.7.1.5/Chest X-Ray	A chest X-ray will be performed at the Screening, ~~Month 12~~Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.	16

Section 9.7.2.1/Blood Phenylalanine Concentrations	Blood samples for Phe concentration measurements will be drawn at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1~~, Table 9.1.2~~: ~~Table 9.1.3 and Table 9.1.4~~). Blood Phe may be collected monthly, rather than weekly, at the discretion of the Investigator and provided that the subject meets the following criteria: • Is receiving a stable dose of rAvPAL-PEG (defined as no dose modifications or interruptions for at least the previous 2 weeks) • Has a stable blood Phe level (defined as Phe between 60-600 µmol/L for at least 2 consecutive weeks) The subject may continue to have monthly, rather than weekly, blood Phe collection provided the following do not occur: • Blood Phe measurement > 600 µmol/L • Blood Phe measurement ≤ 30 µmol/L If either of these conditions occurs, then that subject should return to weekly blood Phe draws until the rAvPAL-PEG dose has been adjusted and the Phe level is again within the protocol-defined target range for at least 2 weeks. ~~In addition, after each administration of study drug, the subject will have a blood Phe measurement by fingerstick as outlined in Table 9.1.1, Table 9.1.2: , Table 9.1.3 and Table 9.1.4 . This may be done by the subject at home.~~	2, 7

Section 9.7.3/Pharmacokinetic Variables	Subjects enrolled in the Substudy will have additional PK sampling performed~~prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG~~. For subjects participating in the Substudy, PK sampling will be performed as follows: • When dosing is stopped, PK sampling will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16 hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours. • When dosing is re-started, PK sampling will be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours.~~For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.~~ • For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose. • For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose. See Table 9.1.2 for additional details.	5, 6
Section 9.7.4/Exploratory Efficacy Variables	Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each ~~week~~month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.	14

Section 9.7.5.2/Antibody Testing	Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, ~~anti-PEG~~ anti-~~PEG~~PAL IgE, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1.3.3). ~~Validated~~I-immunogenicity assays will be ~~used per~~performed at the time points indicated in the Schedule of Events (Table 9.1.1). BioMarin will perform ~~the~~all antibody ~~analysis~~testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.	16
Section 9.7.6/Clinical Laboratory Assessments	All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not ~~any~~ the abnormal result is clinically significant.	16
Table 9.7.6.1/Clinical Laboratory Tests	This table has been updated to reflect changes in this amendment.	16
Section 11.2/Diet Diary	A 3-day diet diary will be issued to subjects for completion and will be brought to ~~each~~ clinic on a monthly basis ~~visit~~ for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.	14
Section 12.2/Screening Visit	• Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray. • Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis) • Complement C_3 and C_4 For subjects who participated in ~~PAL-002 or PAL-004, these assessments may be the same as those used for the Week 16 Visit of PAL-002 or the Week 16 visit of~~PAL-004a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.	1, 9, 16

Section 12.3.1/Day 1 (Week 1)	• Vital signs, ~~including weight~~ • Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the ~~Study~~ Sponsor's Medical Officer) o ~~For subjects enrolling into this study from Study PAL-004, first dose of rAvPAL-PEG must be given in the clinic setting and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.~~	13, 16
Section 12.3.2/Weekly Visits	Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. However, dose modifications must be performed in the clinic. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. For subjects on a stable dose of rAvPAL-PEG (ie, no dose change in the last 2 weeks) and have had a phenylalanine (Phe) level at or below target range (60-600 µmol/L) for 2 consecutive weeks weekly visits may be waived and replaced by a telephone call per discretion of the Investigator but monthly visits are required. • Vital signs, ~~including weight~~ • ~~3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)~~ • ~~For subjects participating in the Substudy, refer to Section 12.3.3~~. • Blood Phe and plasma tyrosine concentration o Blood Phe may be collected monthly, rather than weekly, if certain criteria are satisfied (refer to Section 9.7.2.1). • Weekly telephone call o For those subjects performing self administration and waiving (per Investigator discretion) the weekly visit (refer to Section 12.3.7). o ~~For subjects participating in the Substudy, refer to Section 12.3.3.~~ ~~In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.~~	2, 6, 13, 14

Section 12.3.3/Substudy Visits	For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2: .~~on Day 1 of the Substudy (ie, final dose prior to interruption), Day 8, Day 15, Day 22, and Day 29 (ie, restart administration of study drug) for subjects participating in the Substudy:~~ • ~~Physical examination~~ • ~~Vital signs, including weight~~ • ~~Clinical laboratory tests~~ • ~~Sedimentation rate~~ • ~~Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)~~ • ~~Injection site inspection (predose; postdose on Day 1; during weekly visit on Day 8, 15, 22; and postdose on Day 29)~~ • ~~Assessment of AEs~~ • ~~Concomitant medications~~ • ~~Diet query~~ • ~~3-day diet diary~~ • ~~Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies,)~~ • ~~Blood Phe and plasma tyrosine concentration~~ o ~~When dosing is stopped, sampling for blood Phe assessment only will be performed prior to the final dose and then postdose at 8 hours, 12 hours, 16 hours, 24 hours, 48, hours, 72 hours, 96 hours, 144 hours, 168 hours, and 216 hours.~~	5, 6

Section 12.3.3/Substudy Visits	~~○ When dosing is re-started, sampling for blood Phe assessment only will be performed predose and then postdose at 24 hours, 48 hours, 72 hours, 120 hours, and 168 hours.~~ ~~• For subjects participating in the Substudy, additional PK sampling will be performed upon the final dose prior to the planned dosing interruption and upon re-introduction of rAvPAL-PEG. Refer to Section 9.7.3~~ ~~• Administer study drug (Day 29 only)~~ ~~○ For subjects participating in the Substudy, administration of rAvPAL-PEG will not be performed for approximately 4 consecutive weeks regardless of their dosing regimen (dose level and frequency). After approximately 4 weeks of no dosing, rAvPAL-PEG administration will be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of re-administration must be given in the clinic setting, and a telephone follow-up with the subject must be performed 24 hours later to assess safety issues.~~	5, 6

Section 12.3.4/Monthly Visits (Week 4, 8, 12, etc)	Beginning with Week 4, ~~M~~monthly visits must be performed in the clinic and consist of all weekly activities listed above in Section 12.3.2 as well as the additional ~~and include additional activities and must be performed in the clinic. The following~~ study activities listed below ~~will be performed at the monthly visits beginning with Week 4~~: • ~~Vital signs~~Weight, ~~including weight~~ • Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray. • Chest x-ray (~~Month 12~~Week 48 visit only) • ~~Injection site inspection (previous and current injection site)~~ • ~~Assessment of AEs~~ • ~~Concomitant medications~~ • ~~Diet query~~ • 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next ~~dose of study drug~~monthly visit.) • ~~PK sample~~ • ~~For subjects participating in the Substudy, refer to Section 12.3.3~~. • ~~Blood Phe and plasma tyrosine concentration~~ • ~~Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)~~ • Weekly telephone call o For those subjects performing self administration and waiving (per Investigator discretion) the weekly visit (refer to Section 12.3.7).	13, 14, 16
Section 12.3.4/Monthly Visits (Week 4, 8, 12, etc)	~~o For subjects participating in the Substudy, refer to Section 12.3.3.~~ • ~~In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.~~	13, 14, 16

Section 12.3.5/Quarterly Visits (Week 12, 24, 35, etc)	Beginning with Week 12, ~~Q~~quarterly~~uarterly~~ visits consist of all weekly(Section 12.3.2) and monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below ~~and include additional activities. The following study activities will be performed at the quarterly visits beginning with Week 12:~~ • ~~Vital signs, including weight~~ • ~~Clinical laboratory tests~~ • Complement C_3 and C_4 • ~~Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)~~ • ~~Injection site inspection (previous and current injection site)~~ • ~~Assessment of AEs~~ • ~~Concomitant medications~~ • ~~Diet query~~ • ~~3 day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next dose of study drug.)~~ • ~~PK sample~~ o ~~For subjects participating in the Substudy, refer to Section 12.3.3.~~ • ~~Blood Phe and plasma tyrosine concentration~~ • ~~Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)~~ • ~~Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Study Medical Officer)~~ • ~~For subjects participating in the Substudy, refer to Section 12.3.3.~~	1, 16
Section 12.3.5/Quarterly Visits (Week 12, 24, 35, etc)	~~In addition, 3 days after each administration of study drug, the subject will have a blood Phe measurement by fingerstick. This may be done by the subject at home.~~	

Section 12.3.6/Interim Dosing Visit	~~• Vital signs, including weight~~ • Administer study drug ~~• Blood Phe measurement by fingerstick~~ ~~○ This may be done by the subject at home.~~	7, 16
Section 12.3.7/Weekly Telephone Visit	Subjects who are administered rAvPAL-PEG at home and/or have been approved to self administered rAvPAL-PEG, should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit: • Injection-site self inspection (previous and current injection site) • Assessment of AEs • Concomitant medications • Confirmation of drug administration	16
Section 12.3.8/Unscheduled Hypersensitivity Reaction Visit	~~• Perform immediately after reaction if possible.~~	3, 16
Section 12.3.8/Unscheduled Hypersensitivity Reaction Visit	Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 8 weeks. If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing will not resume until review of the case with the Investigator, Sponsor Medical Officer and an allergist/immunologist ~~Following completion of the Unscheduled Hypersensitivity Reaction Visit assessments, the subject may resume treatment with study drug per the discretion of the Investigator in consultation with the Sponsor's Medical Officer. Subjects who have had an IgE-mediated reaction must be discontinued from further study drug administration. Subjects who do not have an IgE-mediated reaction may resume study drug administration under close monitoring; subjects must be premedicated orally with acetaminophen and/or antihistamines 1 hour prior to study drug dosing for the remainder of the study~~ (refer to Section 9.1.1.3.3).	3, 16

Section 12.4/Early Termination Visit	• Vital signs, ~~including weight~~ • Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.	13, 16
Section 12.5/Final Follow-up Visit	• Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.	16
Section 14.3/Pharmacokinetic Analysis	Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should data become available from ~~PAL-002 or PAL-004~~ previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.	16
Section 14.3.1/Pharmacokinetic Substudy Analysis	For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.	5
Section 14.5/Determination of Sample Size	Subjects who participated in ~~PAL-002 or PAL-004~~ previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.	16

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria (PKU)
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Officer:	[redacted], MD [redacted], Clinical Sciences BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Up to 86 months or until study is terminated
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg or 375 mg/week
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011
Date of Amendment 4:	June 7, 2012
Date of Amendment 5:	February 28, 2014

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 5

Date: February 28, 2014

RATIONALE AND SUMMARY OF CHANGES

The protocol for Study PAL-003 is being amended to make the following changes:

1. Study Duration: The PAL-003 study duration has been extended an additional 26 months to allow continued access to rAvPAL-PEG for subjects who do not qualify for participation in Study 165-302 and to further characterize the long-term safety and tolerability of rAvPAL-PEG in this patient population.
2. Subject Population: The study population has been revised to only include subjects who are on a stable rAvPAL-PEG dosing regimen of <70 mg/week or >350 mg/week. This study is intended to provide long-term access to rAvPAL-PEG until study termination or regulatory approval for subjects who are not eligible to transition into the pivotal, placebo-controlled, Phase 3 study, 165-302. Subjects who are on a stable rAvPAL-PEG dose regimen of ≥ 70 mg/week to ≤ 350 mg/week and do not transition to 165-302 will be withdrawn from this study.
3. Consultation by Sponsor's Medical Officer for Diet and Dose Modifications: Review and approval of diet and dose modifications by the sponsor's medical officer is no longer required for this study, except for dose increases >350 mg/week. Significant modifications to dose and/or diet are not expected as subjects are generally on a stable maintenance rAvPAL-PEG dose regimen and are no longer performing dose titration.
4. Observation Time following Dose Increases or Dose Interruption: The observation period following an increase in rAvPAL-PEG dose or re-introduction of rAvPAL-PEG following a dose interruption has been reduced to 30 minutes as longer periods of observation is have not proven to be useful.
5. Clinic Visits: Clinic visits have been reduced from weekly to monthly because it is anticipated that rAvPAL-PEG dose adjustments will be infrequent (subjects are on a maintenance dose regimen) and to lessen patient burden. However, weekly telephone contacts will continue to be performed. Also, blood sampling for PK and immunogenicity assessments has been revised from monthly to every 12 weeks to better align with the long-term needs of this study and to lessen patient burden.

6. Unscheduled Hypersensitivity Reaction Visit: Assessments of urine/albumin creatinine ratio and urine N-methyl histamine have been added and sedimentation rate has been removed to align with the Hypersensitivity Reaction visit assessments performed in the Phase 3 studies. In addition, the window for the Hypersensitivity Reaction Visit has been changed to within 24 hours of event onset to enable timely assessment of hypersensitivity markers.
7. Immunogenicity Testing: Antibody testing has been clarified. Anti-PAL immunoglobulin E (IgE) testing is no longer required for this study because there has been no observed association between anti-PAL IgE and hypersensitivity reactions. Anti-PAL-PEG IgE testing will continue to be performed as part of the Hypersensitivity Reaction Visit assessments.
8. Safety Analysis: Details regarding analysis of hypersensitivity adverse events (AEs), including those that result in dose modification, have been added. Also, physical examination results and immunogenicity test results have been added to the safety analysis description.
9. Adverse Event Reporting: The pregnancy reporting period has been clarified as the time from informed consent to 4 weeks after the last dose of study drug. Also, AE reporting for events that are not resolved at the time of study completion has been revised; AE outcomes with resolution dates if applicable should be reported on the appropriate Case Report Form (CRF).
10. Premedication: Subjects who have a hypersensitivity reaction are no longer required to premedicate with acetaminophen and antihistamine for all subsequent doses of study drug. However, premedication prior to study drug administration is encouraged.
11. Return to Dosing following Hypersensitivity Reactions: Return to dosing following a hypersensitivity reaction should be based on investigator assessment in consultation with the Sponsor's Medical Officer.
12. Pharmacokinetic Substudy: The sample size for the PK Substudy has been clarified as up to 6 subjects.
13. Investigational Product: rAvPAL-PEG information has been updated to include information regarding the addition of 20-mg vials and prefilled syringes with rAvPAL-PEG.
14. Clinical Background Information: Results from the Phase 2 studies PAL-002, PAL-004, and PAL 165-205 have been updated to align with information provided in the Phase 3 studies.
15. Schedule of Events and Study Procedures: The Schedule of Events and the Study Procedures sections have been updated to reflect the changes to this amendment.
16. Sponsor's Medical Officer: The Medical Officer information has been updated.

17. Administrative Changes: Additional minor changes have been made to improve clarity and consistency.

Specific revisions since the completion of the last protocol amendment (dated 07JUN2012) to the text of each section, including the Synopsis, are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

STUDY DESIGN AND PLAN:

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have

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had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. The first dose will be administered in the clinic, and the subject must be observed for 30 minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

- The subject is discontinued from the study at the discretion of the Investigator.
- The subject has completed the study through the Month 86 visit.
- The study is terminated.
- The study drug receives marketing authorization.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

Subjects who participate in this study will be required to transition into a Phase 3 study (165-302) if weekly doses of rAvPAL-PEG are between 70 mg/week and 350 mg/week (inclusive) provided that doses of rAvPAL-PEG have been stable (ie, no major change in dose for at least 4 weeks). Subjects who meet these criteria must be transitioned from PAL-003 to Study 165-302 provided they meet the 165-302 study eligibility criteria.

Subjects who are eligible for Study 165-302 but do not transition into 165-302 will be withdrawn from this study.

The PAL-003 study will continue until BMN-165 is approved by the Food and Drug Administration (FDA) for marketing in the United States or until the sponsor terminates further development of this drug for the treatment of PKU.

<u>PAL-003 Substudy</u>

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed.

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Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower, more

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

frequent dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be reduced.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

Safety Assessment Criteria:

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Sponsor's Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

Stopping Criteria:

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will be evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

NUMBER OF SUBJECTS PLANNED:

Up to 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.

5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from a previous rAvPAL-PEG study.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PE per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PE per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Or

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Fixed Dosing

Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects should convert their weight-based dose using the following table:

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 10 mg/ml concentration (ml) (vial)	Vol for 15 mg/ml concentration (ml) (vial)[a]
0.03	2.5	0.25	0.17
0.06	5	0.5	0.33
0.12	10	1	0.67
0.25	20	2	1.33
0.5	40	4	2.67
1.0	75	7.5	5
2.0	150	15	10
3.0	225	22.5	15
4.0	300	30	20
5.0	375	37.5	25

[a] Applies to both 1.0 and 1.3 ml withdrawal.

DURATION OF TREATMENT:

Up to 86 months.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG,

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, and anti-PEG-PAL IgE) will be assessed.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in a previous rAvPAL-PEG study may be enrolled into this study.
No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics for vital signs, physical examination results, ECG results, X-ray results, and

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the Statistical Analysis Plan [SAP]) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

Details regarding exploratory analyses will be provided in the SAP.

Substudy Analysis:

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

3 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

[redacted]

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular

DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury

NAb	neutralizing antibodies
NCI	National Cancer Institute
NOAEL	no observable adverse effect level
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Pharmacovigilance Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to

normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively, and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given ≥ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG

(Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.

7.2.1 Phase 1 Study, PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.
- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002, PAL-004, and 165-205

Based on results from the Phase 1, single-dose study, the Phase 2 studies have been designed to evaluate rAvPAL-PEG at various doses and dosing regimens to safely achieve and maintain blood Phe reductions in subjects with PKU. Currently, there are three ongoing Phase 2 studies (PAL-002, PAL-004, 165-205); an overview of each study is presented in Section 7.2.2.1 (PAL-002), Section 7.2.2.2 (PAL-004), and Section 7.2.2.3 (165-205).

7.2.2.1 Study PAL-002

Study PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria) was initiated in September 2009 and has completed enrollment. As of 10 September 2012, 40 subjects have been enrolled and 37 have completed the study. PAL-002 enrolled 11 previously exposed subjects from the single-dose study, PAL-001, and 29 subjects who were naïve to rAvPAL-PEG. Thirty-three of the 37 subjects who completed PAL-002 enrolled into this open-label extension study (PAL-003). Two subjects discontinued from the study due to lost to follow-up, relocation, or other reasons, and 1 subject discontinued from the study due to an AE (skin reaction).

The primary objective of this study was to evaluate the effect of multiple doses of rAvPAL-PEG (ranging from 0.001 mg/kg/week to 1.0 mg/kg/week) on blood Phe concentrations in subjects with PKU with up to 16 weeks of treatment. The secondary objectives of the study were to evaluate the safety and tolerability of SC injections of multiple doses of rAvPAL-PEG, to evaluate the immune response to rAvPAL-PEG, and to evaluate the PK profile of rAvPAL-PEG in subjects with PKU.

The PAL-002 study design consists of two parts. In Part 1, rAvPAL-PEG was administered as a once weekly fixed, low-dose induction regimen for 8 weeks. In Part 2, the rAvPAL-PEG dose was titrated upwards; subjects received adjustable dose increases for up to 8 weeks to achieve a target blood Phe concentration of 600 µmol/L. The doses and dosing schedules were revised with a series of protocol amendments to incorporate the information gained during conduct of this early, open-label, multiple-dose study.

A wide range of doses was planned for the PAL-002 study, beginning with doses as low as 0.001mg/kg/week. However, no substantial reductions in blood Phe level were observed in the majority of subjects who were administered rAvPAL-PEG in the initial four cohorts at doses of 0.001, 0.003, 0.01, and 0.03 mg/kg administered once per week. The absence of

appreciable Phe reduction after 16 weeks of treatment in this range of doses led to a decision to amend the study protocol to include a cohort with a higher starting dose of 0.1 mg/kg/week, which had been previously shown to decrease blood Phe levels to approximately 600 µmol/L in the single-dose, Phase 1 study (PAL-001). After the first 2 weeks of dosing at 0.1 mg/kg/week, transient Phe reduction was observed in this subset of subjects. However, this dosing regimen was accompanied by mild to moderate hypersensitivity reactions primarily after the second weekly dose, suggesting that additional exploration of dosing regimens would be required.

In summary, preliminary results from PAL-002 demonstrated that doses below 0.1 g/kg administered once per week were not effective in reducing Phe levels. While transient reduction of Phe was apparent at doses of at least 0.1 mg/kg given once per week, this dose regimen was associated with a high incidence of systemic hypersensitivity reactions, typically following the second weekly dose. Further escalation above this dose of 0.1 mg/kg/week would be required to sustain the effect on Phe levels over time. Increased doses would require spreading dose administration over several days per week, as once-weekly administration of higher doses would not be practical due to the large volume of study drug. Therefore, additional dosing regimens would need to be explored in a subsequent study (PAL-004).

Once subjects completed Study PAL-002, they were eligible to enroll into this open-label extension study, PAL-003 to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.2 Study PAL-004

PAL-004 (An Open-Label Study to Evaluate the Safety, Tolerability, and Efficacy of Subcutaneous Dose Levels of rAvPAL-PEG Administered Daily in Subjects With Phenylketonuria) was initiated in April 2011 and has completed enrollment. As of 10 September 2012, 16 subjects have been enrolled, 15 subjects have completed the study, and 15 subjects have enrolled into this study (PAL-003). One subject withdrew consent from continued participation prior to study completion. All subjects enrolled in this study were naïve to rAvPAL-PEG exposure.

The objective of this study was to determine if daily administration (defined as 5days/week) of rAvPAL-PEG at dose levels of 0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day was safe and effective in reducing and maintaining blood Phe concentrations to 600 µmol/L in subjects with PKU. The starting dose was 0.4 mg/kg, administered 5 days/week. A total of 12 subjects

were added in sequence and were started on doses of 0.4, 0.2, 0.1, 0.06 mg, or 0.001/kg/day based on incoming safety data. Overall, subjects in PAL-004 who initiated treatment at higher daily doses of 0.1 to 0.4 mg/kg/day achieved immediate and substantial reduction of blood Phe levels, but dosing had to be temporarily reduced or interrupted due to the onset of systemic hypersensitivity reactions at approximately Day 10 of dosing. Phe reductions did not persist in the setting of temporary dose reduction or interruption, but Phe levels generally improved if higher dose levels were reinstated.

To prevent the onset of hypersensitivity reactions that were temporally associated with the onset of anti-drug IgM responses, an additional dosing strategy was assessed in PAL-004; 4 subjects initiated dosing at a low dose of 0.001mg/kg/day or 0.06mg/kg/day for 5 consecutive days and then dosing was suspended for 2 weeks. Dosing was restarted on Day 21 at the initial dose followed by dose titration to target Phe levels. Subjects administered this dosing regimen had similar efficacy, but the incidence of hypersensitivity reactions appeared to be similar to that of subjects who did not have the planned dose interruption, suggesting little advantage with this alternate induction regimen.

Experience from PAL-004 indicated that initiating daily rAvPAL-PEG dosing with relatively high daily doses was not sustainable due to the onset of hypersensitivity reactions that occurred approximately 9 to 12 days after the start of administration, nor was the alternate dosing regimen (2 week drug holiday) well tolerated or effective in Phe reduction. In addition, the daily treatment regimen did not reduce the time to achieve target blood Phe levels seen in previous studies. This study indicated that to improve tolerability, rAvPAL-PEG should be initiated with an induction period at doses substantially lower than 0.4mg/kg, followed by upward dose titration toward target Phe levels.

Once subjects completed Study PAL-004, they were eligible to enroll into this open-label extension study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.3 Study 165-205

Study BMN 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy, and Tolerability of Subcutaneously Administered rAvPAL-PEG in Subjects With PKU for 24 Weeks) was initiated in May 2012. Data from ongoing Studies PAL-002, PAL-004, and PAL-003 suggested that a 4-week treatment course with weekly dosing at a fixed low dose (induction), followed by a weekly 2-fold, upward titration of rAvPAL-PEG to approximately 10-fold higher than the initiation dose (maximum of 375 mg/week) is

effective and well tolerated. The objective of this study was to further assess this dosing regimen. Two rAvPAL-PEG dosing regimens were to be explored in this study: weekly low-dose induction (2.5 mg/week for 4 to 8 weeks), followed by a period of upward dose titration towards a target Phe level (600 µmol/L) followed by maintenance dosing at that level through the 24 week study duration (Group 1). The other dosing regimen planned for this study (Group 2) involved administration of a single bolus dose of 8 mg, followed by a treatment holiday of at least 3 weeks duration followed by resumption and escalation of study drug administration following a pattern similar to that employed in Group 1. A total of 24 subjects were enrolled into 165-205. In addition to confirming an effective, well-tolerated dose regimen, this study incorporated non-weight-based dosing, starting with dose administration once per week at a low dose (2.5mg) with gradual escalation and conversion of dose administration to 5x/week dosing for chronic maintenance therapy.

Once subjects have completed Study 165-205, they are eligible to enroll into this study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week, or into the Phase 3 study, BMN 165-302.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation.

However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

This study is an extension of previous rAvPAL-PEG Phase 2 studies. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from previous rAvPAL-PEG studies will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to Polyethylene Glycol (PEG)

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Toxicity Due to an Immunologic Reaction

The active drug substance in rAvPAL-PEG is a bacterial protein, and as such, it is expected that it may elicit immune recognition and responses. Epitopes that play a role in the immune response may be rendered inaccessible by PEG (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model, although PEGylation does not eliminate antibody formation. Although PEGylation of rAvPAL may have a significant effect on reducing the immunogenicity of the molecule, it is reasonable to expect that some individuals may exhibit immune responses upon exposure to the drug. These immune responses may be clinically irrelevant, cause symptoms of various degrees, or lead to neutralization of biological activity. Subjects participating in clinical trials will be monitored closely with specific antibody titers sedimentation rates, and complete blood counts (CBCs).

As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in the single-dose, Phase 1 study (PAL-001) and in the repeat -dosing Phase 2 studies (PAL-002, PAL-004, and PAL-003). The dose levels in the repeat-dosing studies range from 0.001 to 5.0 mg/kg/week administered in various frequencies (including 5 days a week). The most clinically significant AEs have been hypersensitivity reactions that have led to dosing

interruptions and reductions. Most of the hypersensitivity reactions have been nonserious and mild-to-moderate in severity.

A total of 4 SAEs have been reported in the previous rAvPAL-PEG studies. Of these 4 SAEs, 3 have been reported as related to rAvPAL-PEG. Two of the study-drug related SAEs occurred in Study PAL-001. Both of these SAEs occurred in subjects in the lower dose cohorts: an SAE of hypersensitivity reaction in a subject in the 0.001 mg/kg cohort and an SAE of anaphylactic reaction (urticaria) in a subject in the 0.01 mg/kg cohort. Neither SAE was severe or resulted in discontinuation from the study, and both of the SAEs resolved. There were no SAEs in subjects in the higher dose cohorts (0.03 mg/kg and 0.1mg/kg) in PAL-001. The third study drug-related SAE (angioedema) occurred in a subject in Study PAL-004. The fourth SAE (urticaria, dehydration) was reported as not related to study drug and occurred in a subject in Study PAL-002. There have been no reports of anaphylaxis for any subject treated with rAvPEG-PAL (as of January 2012).

Subjects who have a systemic clinical reaction at any time during this study may undergo a series of assessments to monitor safety, including assessment of complements, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and IgE antibodies. Reintroduction of rAvPAL-PEG dosing after a reaction will depend on clinical assessments and available laboratory data, such as chemistry and urine studies, to ensure that there has been no end-organ damage prior to resuming treatment with rAvPAL-PEG. As of May 2012, antibody titers, including positive IgE titers, have not been predictive of future clinical reactions when dosing with rAvPAL-PEG. Additionally, subjects may be premedicated to prevent or mitigate risk of hypersensitivity reactions (refer to Section 9.1.1.3.3).

PEG itself is considered nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.), however, antibodies against PEG may form when PEG is bound to compounds. (Harris, 2003, Nat.Rev.Drug Discov.),(Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in any significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.). Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. Subjects in the Phase 2 rAvPAL-PEG studies have developed both anti-PEG IgM and anti-PEG IgG antibodies. Antibody formation may limit a

subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.

7.4.2.1 Systemic Skin Reactions

Two out of 25 subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. One of these events was reported as serious, and the other was reported as nonserious; both events were reported following administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications.

The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the [redacted]. Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. Upon study completion, the subject continued to receive regular doses (every 3 months) of Depo-Provera, and no further reactions were reported. The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist. See Section 7.2.1 for the results from Study PAL-001.

The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. The results from nonclinical studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys indicated the formation of anti-rAvPAL antibody titers that did not correspond with observations of

the formation of anti-rAvPAL antibody titers that did not correspond with observations of injection-site reactions. Quantitation of anti-PEG titers were not assessed in the chronic repeat-dose studies in the rat and monkey. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to, during, and after this study is prohibited as a precautionary measure (refer to Section 9.3.2 and Section 9.4.8).

7.4.2.2 Management of Hypersensitivity Reactions

Hypersensitivity reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of hypersensitivity reactions. Because of the small potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection. Subjects who qualify for self administration of study drug will be provided with emergency resuscitation instructions (refer to Section 9.4 and the Subject Self-Administration Training Materials).

In the event of a hypersensitivity reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of hypersensitivity reaction symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.

- Administration of oral or IV glucocorticoids.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment (eg, acetaminiophen or ibuprofen).
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. An allergy and/or immunology consultation should be sought if necessary. Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.1.1. Detailed instructions for the management of hypersensitivity reactions are provided in the Study Reference Manual.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objective is as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity was observed in a previous rAvPAL-PEG studies.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer. The first dose will be administered in the clinic, and the subject must be observed for 30 minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to clinic monthly for review with the clinical study staff.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self administration. Additional information is provided in the Subject Self-Administration Training Materials.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will generally continue in PAL-003.

In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 9.1.1 and Table 9.1.2. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator.
- The subject has completed the study through the Month 86 visit.
- The study is terminated.
- The study drug receives marketing authorization.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

Subjects who participate in this study will be required to transition into a Phase 3 study (165-302) if weekly doses of rAvPAL-PEG are between 70 mg/week and 350 mg/week (inclusive) provided that doses of rAvPAL-PEG have been stable (ie, no major change in dose for at least 4 weeks). Subjects who meet these criteria must be transitioned from PAL-003 to Study 165-302 provided they meet the 165-302 study eligibility criteria.

Subjects who are eligible for Study 165-302 but do not transition into 165-302 will be withdrawn from this study.

The PAL-003 study will continue until BMN-165 is approved by the FDA for marketing in the United States or until the sponsor terminates further development of this drug for treatment of PKU.

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2).

The Schedule of Events and PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2

Table 9.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
Informed consent	X									
Medical history, including allergy history, and demographics	X									
Physical examination[e]	X	X			X		X		X	
Vital signs[e]	X	X		X		X			X	X
Weight	X			X						
12-lead ECG	X						X		X	
Clinical laboratory tests[f]	X			X			X		X	X[g]
Complements C_3 and C_4[h]	X				X					X
Sedimentation rate		X			X		X		X	
Chest x-ray	X			X (Week 48 visit only)			X		X	
Urine pregnancy test[i]	X	X		X			X		X	

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
Injection-site inspectionj[i j]		X (postdose)		X		X	X	X	X	X
Adverse events[j, k]	X	X		X		X	X	X	X	X
Weekly phone call to self admin participants only (to assess AEs, Inj site reactions, concomitant medications)			X					X	X	
Concomitant medications[j]	X	X		X		X	X	X	X	X
Diet query	X	X		X			X		X	
3-Day diet diary[l]				X			X		X	
Serum antibodies [m]		X			X		X		X	X
Plasma Phe and plasma tyrosine[n]	X	X		X[m]			X		X	
Urine albumin/creatinine ratio										X
Urine N-methyl histamine										X

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self Administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures	Up to daily	1 week after final dose		4 weeks after final dose	
Plasma PK sample [m]		X			X		X		X	
Administer study drug[o]		X		X		X		X		
Skin biopsy (optional; affected and not affected area)										X
Serum tryptase level [p]										X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] A separate Screening visit for this study is required only if the time between completion of the previous rAvPAL-PEG study and enrollment into PAL-003 is greater than 28 days.

[b] Monthly visits must be performed in the clinic.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Refer to Section 9.1.1.3.3 and Section 12.3.6.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis. Urine microscopy will be performed if any urinalysis results are positive for hematuria. Refer to Table 9.7.6.1.

[g] Subjects who have a systemic reaction or a large local reaction not contiguous with the injection site should be assessed for CRP, CH50, C_1, C_3, and C_4 within 24 hours of the reaction.

[h] Complement C_3 and C_4 will be collected at the Screening Visit and quarterly. Additional complement testing will be performed and as needed to resolve previous abnormal test results.

[i] If positive or equivocal, perform serum pregnancy test.

[j] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm).

[j] Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

[k] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug to the final study visit.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.

[m] Sampling should be performed predose.

[n] Samples should be drawn at least 2.5 hours after a meal. At the Investigator's discretion, blood Phe may be collected more often should more frequent monitoring of blood Phe levels be clinically warranted

[o] Dosing is up to 5.0 mg/kg/week or 375 mg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may be re-evaluated.

[p] Mandatory for subjects who have a systemic reaction. Take sample immediately after reaction if possible.

Table 9.1.2: PK Substudy Dosing Regimens

Treatment Frequency	Plasma PK Sampling	Example
Subjects who are administered study drug once per week	Pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours when possible), and pre-dose of the next weekly dose	If dosed on Monday: Obtain PK sample pre-dose when possible – pre-dose Monday, Tuesday, Wednesday, Thursday, Friday, and pre-dose the following Monday. No further PK substudy draws.
Subjects who are administered study drug two or three times per week	Pre-dose and every 24 hours during the longest period between doses, when possible, and pre-dose of the next dose.	If dosed on Monday, Thursday, and Friday: Obtain PK sample every 24 hours during the longest period between doses: Pre-dose Monday, Tuesday, Wednesday, and pre-dose Thursday. No further PK substudy draws.
Subjects who are administered study drug four to seven times per week.	Pre-dose and every 12 hours during the longest period between doses, when possible, then pre-dose of the next dose.	If dosed Monday through Friday: Obtain PK sample every 12 hours during the longest period between doses (Friday to Monday): pre-dose Friday, 12 hours post-dose Friday, Saturday (24 and 36 hours post dose), Sunday (48 and 60 hours post-dose), and pre-dose the following Monday. No further PK substudy draws.

9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG

Subjects who qualify for self administration of study drug will be provided with information and instruction with regard to management of local and systemic reactions (refer to the Subject Self-Administration Training Materials).

9.1.1.1 Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product

Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from a previous rAvPAL-PEG study are excluded from participation in this study. Subjects who have had a previous local skin reaction to rAvPAL-PEG in a previous rAvPAL-PEG study are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing for the remainder of the study. The premedication dosage will be standard. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable). Subjects may develop systemic, large local skin, or local skin reactions after enrollment in PAL-003. Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.

9.1.1.2 Definition of Reaction and Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously

During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows:

Local skin reaction:

- Skin signs or symptoms in 1 affected primary location, ie, hives, wheals, or swelling or an area of erythema, redness, induration, pain, or itching at or near the site of injection.

Large local skin reaction:

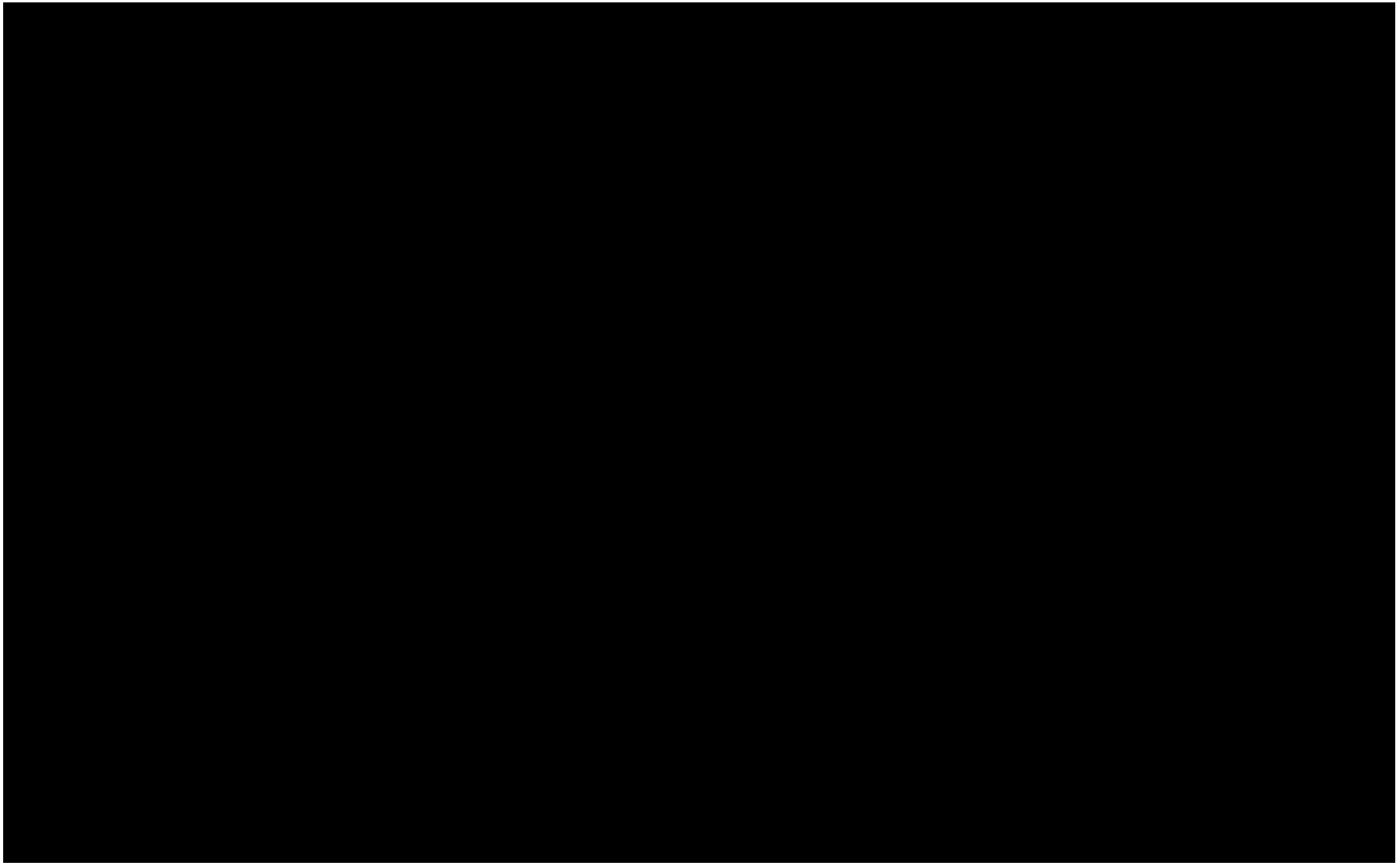
- Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site.

Systemic reaction (including generalized skin symptoms):

- Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological; and/or a fever attributed to treatment with rAvPAL-PEG ($\geq$ 100.4°F or $\geq$ 38°C).

For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.

[redacted]



9.1.1.3.1 Local Skin Reactions

All subjects who experience a local skin reaction during this study may be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Recommended premedications are acetaminophen and/or non-sedating antihistamine. The dosage will be standard. Subjects must be accompanied by a designated driver (if applicable) because antihistamines can cause drowsiness. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2).

If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from study treatment.

9.1.1.3.2 Large Local Skin Reactions

Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects will be encouraged to premedicate orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site

Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. For the remainder of the study, subjects will be encouraged to premedicate orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site

Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction (refer to Section 9.1.1.3.3). An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.

Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.2.2).

9.1.1.3.3 Systemic Reactions

Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG must stop further administrations of rAvPAL-PEG and must immediately return to the clinic for safety assessments. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 24 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Reaction Visit): serum antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti rAvPAL-PEG IgE, anti-rAvPAL-PEG neutralizing antibodies); serum tryptase level (it is recommended that this sample be drawn immediately after reaction); urine albumin/creatinine ratio; urine N-methyl histamine; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1.

Following the reaction, subjects will be required to complete the assessments of the Unscheduled Hypersensitivity Reaction visit, including assessment of IgE. If a subject presents with a clinical diagnosis of anaphylaxis, further dosing will be held while laboratory evaluation of IgE, as part of the Unscheduled Systemic Reaction Visit, is performed. Subjects may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 1 week.

rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects will be encouraged to premedicate orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section 9.1.2. Subjects will be encouraged to premedicate orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).

9.1.2 Dose Modifications

9.1.2.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.

- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

9.1.2.2 Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1.2). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower, more frequent dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be reduced.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

9.1.3 Safety Assessment Criteria

If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Sponsor's Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.

9.1.4 Stopping Criteria

If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.

5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the Sponsor's Medical Officer. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG or its excipients, including hypersensitivity reactions that necessitated early termination from a previous rAvPAL-PEG study.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study treatment should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study treatment include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in their previous rAvPAL-PEG study. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose that was administered upon completion of that study provided that there was no interruption in dosing. rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increased per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects may be eligible to self administer study drug if he or she meets the following criteria:

- The subject is on a stable dosing regimen for 2 weeks (ie, the subject has demonstrated a blood Phe level within 60-600 µmol/L).
- The subject has not experienced any CTCAE Grade 3 or higher adverse event.

- The subject has not experienced any hypersensitivity reaction to rAvPAL-PEG for at least 4 weeks.
- The subject has no cognitive impairments that may increase the safety risk of self administration per the assessment of the PI.
- The subject has been approved for self administration of study drug by the Sponsor's Medical Officer.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with epinephrine and has been trained on when and how to administer it.

Qualified study site personnel will train each eligible subject on all procedures for self administration of study drug under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self administer a dose at home. The subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 9.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials.

Subjects who are eligible for self administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.
- How to safely use and dispose of syringes used for injections of study drug.
- How to use a new syringe and vial every time drug is administered.
- How to care for their injection site after an injection of study drug.

- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine.
- Who to contact at the study site in case of an emergency.

The PI or the Sponsor's Medical Officer may request that self administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplying the study drug to subjects who qualify for self administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PE per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

Drug product packaging will be identified with the lot number and expiration date, and will be provided in a box labeled with the study number.

9.4.3 Storage

IP must be stored at 5 ±3° C (41 ± 5° F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose).

For subjects receiving weight-based dosing, dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement

obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1.

Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 10 mg/ml concentration (ml) (vial)	Vol for 15 mg/ml concentration (ml) (vial)[a]
0.03	2.5	0.25	0.17
0.06	5	0.5	0.33
0.12	10	1	0.67
0.25	20	2	1.33
0.5	40	4	2.67
1.0	75	7.5	5
2.0	150	15	10
3.0	225	22.5	15
4.0	300	30	20
5.0	375	37.5	25

[a] Applies to both 1.0 and 1.3 ml withdrawal.

It is preferable that the injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). The number of injections required for an individual weighing 80 kg (about 176 pounds) is provided in Table 9.4.4.2 as an example. Note this table is for example purposes only. Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff, other qualified and trained study personnel, or qualified subjects.

Information for dosing of subjects who enroll into this study after completing previous rAvPAL studies is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self administration are provided in the Subject Self-Administration Training Materials.

Table 9.4.4.2: Example of Number of Injections Required For an Individual Weighing 80 kg

Dose Group (mg/kg)	Weight (kg)	Volume of Study Drug (mL)[a]	No. of Injections[b]
0.001	80	0.8	1
0.003	80	0.6	1
0.01	80	0.8	1
0.03	80	0.2	1
0.06	80	0.5	1
0.1	80	0.8	1
0.3	80	2.4	2
0.6	80	4.8	2 or 3
1.0	80	8.0	4

aStudy drug is supplied at a concentration of 10 mg/mL and is then diluted (where necessary for injection) by differential amounts as specified in the PAL-003 Pharmacy Manual. Volume to be injected is calculated based on a starting volume.

[b]Number of injections per dose is dependent upon site policy for SC injections. The number of injections may vary at the discretion of the Investigator.

Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may need to be re-evaluated (refer to Section 9.4.10).

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in their previous rAvPAL-PEG study.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self administer study drug at home if approved by the Sponsor's Medical Officer and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the Sponsor's Medical Officer must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be encouraged to be premedicate with acetaminophen and/or non-sedating antihistamines (refer to Section 9.1.1). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior systemic reaction to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.1.1).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for dosing of subjects who enroll into this study after completing previous rAvPAL-PEG studies is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator. Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30 minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1 during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study treatment per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately

prior to their next monthly study visit.The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a]	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Pregnancy test (urine) and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1
Urinalysis	Central laboratory	9.7.6

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and

neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn monthly, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1).

Blood Phe may be collected more often than monthly at the discretion of the Investigator should more frequent monitoring of blood Phe levels be clinically required.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed. For subjects participating in the Substudy, PK sampling will be performed as follows:

- For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.

- For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose.
- For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

See Table 9.1.2 for additional details.

BioMarin will perform the analysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.4 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to

Section 9.1.1.3.3). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1).

BioMarin will perform all anti-body testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Phenylalanine
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Sedimentation rate[a]
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Spot urine albumin/creatinine ratio	**Additional Unscheduled Hypersensitivity Reaction Visit Tests[b]**
Total protein		Nitrite	CH50
Calcium		Urobilinogen	C_1, C_3, C_4
Sodium		Hemoglobin	Serum tryptase level[b]

Blood Chemistry	Hematology	Urine Tests[a]	Other
Potassium		Bilirubin	CRP
Glucose			Urine N-methyl histamine
			Urine albumin/creatinine ratio
Uric acid			**Complement Testing[c]**
CO_2			C_3
Chloride			C_4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory.

[b] Perform immediately after reaction if possible.

[c] Complement C_3 and C_4 to be drawn at the Screening Visit and then quarterly or as needed to resolve abnormal test results.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study period during which all non-serious AEs and SAEs will be reported begins after informed consent is obtained and the first administration of study drug and continues until 4 weeks following the last administration of study drug or the last visit of the treatment period (refer to Section 12). After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. Outcome of AEs (and resolution dates) should be documented on the appropriate CRF page(s) and in the subject’s medical record unless the subject is lost to follow-up or it has been determined that the study treatment or study participation is not the cause of the AE.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to

seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)
4	Life threatening or debilitating: consequences; urgent intervention indicated[b]
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	*Description*
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
    - Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Is an important medical event or reaction.

The reporting period for SAEs begins after informed consent is obtained, and continues until 4 weeks following the last administration of study drug or End of Treatment Visit.

All SAEs, whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form to BioMarin Pharmacovigilance (BPV). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the subject's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution to BPV in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the

competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (i.e., modified dose amount or frequency not defined in protocol).
- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.5 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the Sponsor's Medical Officer any AEs for which the issue of seriousness is unclear or questioned. Contact information for the Sponsor's Medical Officer is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to clinic on a monthly basis for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis)
- Complement C_3 and C_4
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week

unless otherwise noted. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Injection-site inspection (postdose)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator)

12.3.2 Weekly Telephone Visit

Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:

- Injection-site self inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Confirmation of dose level, frequency, and study drug administration

12.3.3 Substudy Visits

For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2.

12.3.4 Monthly Visits (Week 4, 8, 12, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 4, monthly visits must be performed in the clinic. The following study activities will be performed:

- Vital signs
- Weight
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray.
- Chest x-ray (Week 48 visit only)
- Injection-site inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.)

12.3.5 Quarterly Visits (Week 12, 24, 36, etc)

Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 12, quarterly visits consist of all monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below:

- Physical examination
- Complement C_3 and C_4
- Sedimentation rate
- PK sample
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)

12.3.6 Unscheduled Hypersensitivity Reaction Visit

Subjects who have a systemic reaction, including a generalized skin reaction, or a large local skin reaction that is not contiguous to the injection site after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 24 hours) the reaction.

- Vital signs
- Injection-site inspection
- Clinical laboratory tests
- Urine/albumin creatinine ratio
- CRP, CH50, C1, C3, and C4
- Urine N-methyl histamine
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies)

- Serum tryptase level
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction)
- Assessment of AEs
- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 8 weeks.

12.4 Early Termination Visit

The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who terminate from study treatment early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications

- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

12.5 Final Follow-up Visit

The final follow-up (F/U) Visit will occur 1 week after the final dose of study drug.

The following study activities will be performed at the F/U Visit:

- Physical examination
- Vital signs, including weight
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Injection-site inspection (previous injection site)
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Final Follow-up Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF).

The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statisticsof vital signs, physical examination results, ECG results, and X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration

(T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should data become available from previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the Sponsor's Medical Officer.

14.3.1 Pharmacokinetic Substudy Analysis

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the SAP) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis.

Details regarding exploratory analyses will be provided in the Statistical Analysis Plan.

14.5 Determination of Sample Size

Subjects who participated in previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Assess the effect and relevance of new external evidence.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.4.
- Make recommendations to the BioMarin concerning continuation or termination of the study or other study modifications based on observations.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment, unless the executed Clinical Trial Agreement between BioMarin and the institution of the investigator specifies otherwise. If the subject has followed the Investigator's instructions, BioMarin may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the

clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003, Amendment 5

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

On behalf of BioMarin, I confirm that BioMarin, as a sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

[redacted] 28 FEB 2014

Medical Monitor Signature Date

Printed name: _____ [redacted] _____

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following table summarizes the revisions made to the protocol and relates the changes to the appropriate rationale (see pages 2-4). Text that has been added or inserted is indicated by underlined font, and deleted text is indicated by ~~strikethrough font~~.

Section No./Title	Revision	Rationale
Global change: Sponsor's Medical Officer	[redacted], MD	16
Section 2/Synopsis, Study Rationale	….This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess ~~whether long-term dosing~~the long-term safety and efficacy of rAvPAL-PEG ~~is safe and can maintain reduced blood Phe concentrations~~ in PKU subjects. ….Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator~~in consultation with the Sponsor's Medical Officer~~. Diet will be monitored using a diet diary. A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in ~~a minimum of~~up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive ~~weeks~~ measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001….	2, 3, 12

Section 2/Synopsis, Study Design and Plan	This is a long-term extension of rAvPAL-PEG Phase 2 studies in ~~approximately~~ up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies ~~PAL-002 and PAL-004~~ (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study. …. The first dose will be administered in the clinic, and the subject must be observed for 30~~-60~~ minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator ~~in consultation with the Sponsor's Medical Officer~~ and must be performed under the supervision of the Investigator ~~in consultation with the Sponsor's Medical Officer~~. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose ~~(subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks)~~. Doses will be evaluated on an individual basis. A subject will continue in PAL-003 until one of the following occurs: • The subject has completed the study through the Month 8~~60~~ visit. • The study drug receives marketing authorization.	2, 3, 4, 12

Section 2/Synopsis, Study Design and Plan	Subjects who participate in this study will be required to transition into a Phase 3 study (165-302) if weekly doses of rAvPAL-PEG are between 70 mg/week and 350 mg/week (inclusive) provided that doses of rAvPAL-PEG have been stable (ie, no major change in dose for at least 4 weeks). Subjects who meet these criteria must be transitioned from PAL-003 to Study 165-302 provided they meet the 165-302 study eligibility criteria. Subjects who are eligible for Study 165-302 but do not transition into 165-302 will be withdrawn from this study. The PAL-003 study will continue until BMN-165 is approved by the Food and Drug Administration (FDA) for marketing in the United States or until the sponsor terminates further development of this drug for the treatment of PKU. PAL-003 Substudy A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in ~~a minimum of~~up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive ~~weeks~~measurements and for whom no further dose modifications are planned.	2, 3, 4, 12

Section 2/Synopsis, Dose Modifications	Dose Increase Methodology: Each subject's dose may be modified based on the decision of the Investigator~~, in agreement with the Sponsor's Medical Officer~~. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations. An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows. • When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg~~)~~ or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination ~~in consultation with the Sponsor's Medical Officer~~ based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor. • The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg ~~Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit)~~or 375 mg/week. Dose increases >350 mg/week require consultation with the sponsor's medical monitor. • When a dose is increased, the subject must be observed for 30~~-60~~ minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.	2,3,4

Section 2/Synopsis, Dose Modifications	Dose Decrease Methodology ~~All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer.~~ Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for ~~safety (ie,~~ hypophenylalanemia or any adverse event that may be improved with a lower, more frequent dose~~)~~. A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required ~~to attain the target Phe concentration~~in response to safety, dosing frequency may also be reduced. If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L~~as measured by serum Phe levels for a minimum of 1 month~~), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).	2,3
Section 2/Synopsis, Number of Subjects Planned	~~Approximately~~Up to 100 subjects.	17

<table>
<tr>
<td>Section 2/Synopsis, Investigational Product, Dose, Route and Regimen</td>
<td>
The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self administration:~~rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg or rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.~~

rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PE per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PE per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or

rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

<table>
<tr><th>Prefilled Syringe</th><th>Volume</th><th>Concentration</th><th>Dose</th></tr>
<tr><td>Sku #1</td><td>0.5 mL</td><td>5 mg/mL</td><td>2.5 mg</td></tr>
<tr><td>Sku #2</td><td>0.5 mL</td><td>20 mg/mL</td><td>10 mg</td></tr>
<tr><td>Sku #3</td><td>1.0 mL</td><td>20 mg/mL</td><td>20 mg</td></tr>
</table>
</td>
<td>13</td>
</tr>
</table>

Section 2/Synopsis, Investigational Product, Dose, Route and Regimen	The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection. ~~Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose than the dose that was administered upon completion of that study provided that there was no interruption in dosing. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the Sponsor's Medical Officer.~~ rAvPAL-PEG doses will be administered SC by either health care professionals or via self administration. The dose level may be increasedor decreased per Investigator determination ~~in consultation with the Sponsor's Medical Officer~~ based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 ~~weeks~~ consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, ~~as agreed by~~per the PI ~~and the Sponsor's Medical Officer,~~ based upon the subject's response to doses. **Fixed Dosing** Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive ~~weeks~~measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive ~~weeks~~ measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects should convert their weight-based dose using the following table: Tabular information regarding fixed doses has been revised.	3, 13
Section 2/Synopsis, Duration of Treatment	Up to 8660 months.	1

Section 2/Synopsis, Statistical Methods	**Safety Analysis**: The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). ~~All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).~~The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented. Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics~~, including clinically significant changes from baseline, of~~ for vital signs, physical examination results, ECG results, ~~and~~ X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point. **Efficacy Analysis**: Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the Statistical Analysis Plan [SAP]) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point. **Exploratory Analysis**: ~~For subjects who have received any study drug in this study with any post-treatment blood Phe concentration measurements and diet diary information, the relationship of dietary Phe intake (per information reported on the subject diet diary), and blood Phe concentration will be explored~~Details regarding exploratory analyses will be provided in the SAP.	8, 17

Section 6, Investigators and Study Administrative Structure	The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's ~~Regulatory Affairs~~Pharmacovigilance Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required….	17
Section 7.2, Previous Clinical Studies	The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience ~~as of JAN2011~~ from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.	17
Section 7.2.2, Phase 2 Studies PAL-002, PAL-004, and 165-205	This section has been updated with results from Studies PAL-002, PAL-004, and PAL 165-205.	14

Section 7.3, Study Rationale	Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator ~~in consultation with the Sponsor's Medical Officer~~. Diet will be monitored using a diet diary. A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in ~~a minimum of~~up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive ~~weeks~~ measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range. This study is an extension of previous rAvPAL-PEG Phase 2 studies. Administration of rAvPAL-PEG will be continued to assess ~~whether long-term dosing~~the long-term safety and efficacy of rAvPAL-PEG ~~is safe and can maintain reduced blood Phe concentrations~~ in PKU subjects.	2, 3, 12
Section 7.4.2, Toxicity Due to an Immunologic Reaction	….Additionally, subjects ~~will~~may be premedicated to prevent or mitigate risk of hypersensitivity reactions (refer to Section 9.1.1.3.3).	10

Section 7.4.2.2, Management of ~~Allergic~~ Hypersensitivity Reactions	~~Allergic~~ Hypersensitivity reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30~~ 60~~ minutes post-injection.	3, 17

Section 9.1, Overall Study Design and Plan	This is a long-term extension of rAvPAL-PEG Phase 2 studies in ~~approximately~~ up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies ~~PAL-002 and PAL-004~~ (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study. …. The first dose will be administered in the clinic, and the subject must be observed for 30~~-60~~ minutes following administration. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator ~~in consultation with the Sponsor's Medical Officer~~ and must be performed under the supervision of the Investigator ~~in consultation with the Sponsor's Medical Officer~~. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis. PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose ~~(subjects who have maintained blood Phe levels to 60-600 µmol/L for at least 2 consecutive weeks and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive weeks)~~. Doses will be evaluated on an individual basis. A subject will continue in PAL-003 until one of the following occurs: • The subject has completed the study through the Month 8~~6~~0 visit. • The study drug receives marketing authorization.	2, 3, 4, 12

Section 9.1, Overall Study Design and Plan	Subjects who participate in this study will be required to transition into a Phase 3 study (165-302) if weekly doses of rAvPAL-PEG are between 70 mg/week and 350 mg/week (inclusive) provided that doses of rAvPAL-PEG have been stable (ie, no major change in dose for at least 4 weeks). Subjects who meet these criteria must be transitioned from PAL-003 to Study 165-302 provided they meet the 165-302 study eligibility criteria. Subjects who are eligible for Study 165-302 but do not transition into 165-302 will be withdrawn from this study. The PAL-003 study will continue until BMN-165 is approved by the Food and Drug Administration (FDA) for marketing in the United States or until the sponsor terminates further development of this drug for the treatment of PKU. A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in ~~a minimum of~~up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive ~~weeks~~measurements and for whom no further dose modifications are planned….	2, 3, 4, 12
Section 9.1, Table 9.1.1, Schedule of Events	This table has been revised to align with changes made within the amendment.	15, 17
Section 9.1.1.1, Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product	Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from a previous rAvPAL-PEG study are excluded from participation in this study. ~~Subjects who experienced a generalized skin rash are included in this category.~~ Subjects who have had a previous local skin reaction to rAvPAL-PEG in a previous rAvPAL-PEG study are eligible to participate in this study.	17

[redacted]	[redacted]	17
Section 9.1.1.3.1, Local Skin Reactions	All subjects who experience a local skin reaction during this study may be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Recommended premedications are acetaminophen and/or non-sedating antihistamine. The dosage will be standard. Subjects must be accompanied by a designated driver (if applicable) b~~B~~ecause antihistamines can cause drowsiness~~, sedating antihistamines may be administered only if the subject is accompanied by a designated driver~~. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.2.2).	17
Section 9.1.1.3.2, Large Local Skin Reactions	….Subjects will be encouraged to premedicate~~d~~ orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses.	10

Section 9.1.1.3.2.1, Large Local Skin Reactions Contiguous to Injection Site	Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. For the remainder of the study, subjects will be encouraged to premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).	10

Section 9.1.1.3.3, Systemic Reactions	….Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 2448 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Reaction Visit): serum antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti rAvPAL-PEG IgE, anti-rAvPAL-PEG neutralizing antibodies); serum tryptase level (it is recommended that this sample be drawn immediately after reaction); sedimentation rate;urine albumin/creatinine ratio; urine N-methyl histamine; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1. …. Subjects who do not have an IgE-mediated reaction may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 1 week. If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing will not resume until review of the case with the Investigator, Sponsor's Medical Officer, and an allergist/immunologist. rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects must will be encouraged to premedicated orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30-60 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).	4,6,7, 10, 11

Section 9.1.1.3.3, Systemic Reactions	…. Subjects ~~must~~ will be encouraged to premedicate~~d~~ orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30~~-60~~ minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).	4,10

Section 9.1.2.1, Dose Increase Methodology	Dose Increase Methodology: Each subject's dose may be modified based on the decision of the Investigator~~, in agreement with the Sponsor's Medical Officer~~. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations. An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows. • When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg~~)~~ or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination ~~in consultation with the Sponsor's Medical Officer~~ based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor. • The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg ~~Subjects who increase their dose frequency will have additional assessments performed (Interim Dosing Visit)~~or 375 mg/week. Dose increases >350 mg/week require consultation with the sponsor's medical monitor. • When a dose is increased, the subject must be observed for 30~~-60~~ minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.	2,3,4

Section 9.1.2.2, Dose Decrease Methodology	Dose Decrease Methodology ~~All dose decreases must be agreed to by both the Investigator and the Sponsor's Medical Officer.~~ Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for ~~safety (ie,~~ hypophenylalanemia or any adverse event that may be improved with a lower, more frequent dose~~)~~. A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required ~~to attain the target Phe concentration~~in response to safety, dosing frequency may also be reduced. If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L~~as measured by serum Phe levels for a minimum of 1 month~~), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).	2,3

Section 9.4, Treatments	…. The dose level may be increased per Investigator determination ~~in consultation with the Sponsor's Medical Officer~~ based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both~~, as agreed to by~~per the PI ~~and the Sponsor's Medical Officer~~, based upon the subject's response to doses. Subjects may self administer study drug at home if approved by the Investigator and the Sponsor's Medical Officer and if adequate training is demonstrated. Subjects may be eligible to self administer study drug if he or she meets the following criteria: • The subject is on a stable dosing regimen for 2 weeks (ie, the subject has demonstrated a blood Phe level within 60-600 µmol/L ~~for a minimum of 2 consecutive weeks~~).	3, 5

Section 9.4.2.1, Product Characteristics and Labeling	The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self administration:~~rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg or rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.~~ • rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PE per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product. Or • rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PE per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL. Or • rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).	13

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

Section 9.4.2.1, Product Characteristics and Labeling	~~The rAvPAL-PEG drug product for SC injection is formulated and supplied as a sterile aqueous (colorless to pale yellow) solution in clear, single-use, type 1 borosilicate glass vials. Each 3 mL vial contains 1.5 ± 0.2 mL to deliver either 10 mg of rAvPAL-PEG per 1 mL (10 mg/mL protein concentration) or 15 mg of rAvPAL-PEG per 1 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1 mL of the drug product.~~ ~~Dilution instructions are provided in a separate instruction manual.~~	13
Section 9.4.4, Directions for Administration	Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, ~~as agreed by~~per the PI ~~and the Sponsor's Medical Officer~~, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose). Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive ~~weeks~~measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive ~~weeks~~measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1. Table 9.4.4.1 has been revised.	3, 13

Section 9.4.8, Prior and Concomitant Medication	….Subjects who have had a prior local skin reaction to rAvPAL-PEG or a PEG-containing product will be encouraged to~~will~~ be premedicate~~d~~ with acetaminophen and/or non-sedating antihistamines (refer to Section 9.1.1). If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.	10
Section 9.4.10, Dose Interruption and Missed Doses	During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator ~~in consultation with the Sponsor's Medical Officer~~. Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30~~-60~~ minutes following administration due to restarting dosing. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1 during any dosing interruption.	3, 4

Section 9.6, Dietary or Other Protocol Restrictions	Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator ~~in consultation with the Sponsor's Medical Officer~~ and must be performed under the supervision of the Investigator ~~in consultation with the Sponsor's Medical Officer~~.	3

Section 9.7.2.1, Blood Phenylalanine Concentration	Blood samples for Phe concentration measurements will be drawn monthly, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1:). Blood Phe may be collected more often than monthly~~, rather than weekly~~, at the discretion of the Investigator ~~and provided that the subject meets the following criteria~~should more frequent monitoring of blood Phe levels be clinically required. • ~~Is receiving a stable dose of rAvPAL-PEG (defined as no dose modifications or interruptions for at least the previous 2 weeks)~~ • ~~Has a stable blood Phe level (defined as Phe between 60-600 µmol/L for at least 2 consecutive weeks)~~ ~~The subject may continue to have monthly, rather than weekly, blood Phe collection provided the following do not occur:~~ • ~~Blood Phe measurement > 600 µmol/L~~ • ~~Blood Phe measurement ≤ 30 µmol/L~~ ~~If either of these conditions occurs, then that subject should return to weekly blood Phe draws until the rAvPAL-PEG dose has been adjusted and the Phe level is again within the protocol-defined target range for at least 2 weeks.~~	5

Section 9.7.5.2, Antibody Testing	Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG~~, anti-PAL IgE~~, anti-PEG IgM, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1.3.3). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1:).	7
Section 9.7.6, Clinical Laboratory Assessments	Table 9.7.6.1 has been updated to reflect changes made within this amendment.	6, 17
Section 10.1, Adverse Events	The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. ~~Resolution~~ Outcome of AEs (and resolution~~with~~ dates) should be documented on the appropriate CRF page(s) and in the subject's medical record unless the subject is lost to follow-up or it has been determined that the study treatment or study participation is not the cause of the AE.	9

Section 10.3, Pregnancy	Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution to BPV ~~on the Pregnancy Follow-up Form~~in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.	9
Section 10.5, BioMarin Pharmacovigilance Contact Information	Name: [redacted], MD Address: 105 Digital Drive Novato, CA 94949 USA Phone: [redacted] Fax: [redacted] E-mail: [redacted]	16
Section 12.3.1, Day 1 (Week 1)	• Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator~~, in agreement with the Sponsor's Medical Officer~~)	3

Section 12.3.2, Weekly Telephone Visits	Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1: . The following should be discussed with the subject during the weekly telephone visit: • Injection-site self inspection (previous and current injection site) • Assessment of AEs • Concomitant medications • Confirmation of dose level, frequency, and study drug administration ~~Weekly visits do not require an in-clinic visit if conducted by a home healthcare professional. However, dose modifications must be performed in the clinic. Subjects must have AEs and concomitant medications assessed and an injection-site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. For subjects on a stable dose of rAvPAL-PEG (ie, no dose change in the last 2 weeks) and have had a phenylalanine (Phe) level at or below target range (60-600 µmol/L) for 2 consecutive weeks - weekly visits may be waived and replaced by a telephone call per discretion of the Investigator but monthly visits are required.~~ ~~The following study activities will be performed at the weekly visits beginning with Week 2:~~ ~~• Vital signs~~ ~~• Injection-site inspection (previous and current injection site)~~ ~~• Assessment of AEs~~ ~~• Concomitant medications~~ ~~• Diet query~~ ~~• PK sample~~	5,15

Section 12.3.2, Weekly Telephone Visits	~~• Blood Phe and plasma tyrosine concentration~~ ~~○ Blood Phe may be collected monthly, rather than weekly, if certain criteria are satisfied (refer to Section 9.7.2.1).~~ ~~• Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator, in agreement with the Sponsor's Medical Officer)~~ ~~• Weekly telephone call~~ ~~○ For those subjects performing self administration and waiving (per Investigator discretion) the weekly visit (refer to Section 9.1.1.3.3).~~	5, 15

Section 12.3.4, Monthly Visits (Week 4, 8, 12, etc.)	Beginning with Week 4, monthly visits must be performed in the clinic. The following study activities will be performed~~and consist of all weekly activities listed above in Section 12.3.2 as well as the additional study activities listed below~~: • Vital signs • Injection-site inspection (previous and current injection site) • Assessment of AEs • Concomitant medications • Diet query • Blood Phe and plasma tyrosine concentration • ~~PK sample~~ • ~~Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)~~ • ~~Weekly telephone call~~ o ~~For those subjects performing self-administration and waiving (per Investigator discretion) the weekly visit (refer to Section 12.3.6.~~	5, 15

Section 12.3.5, Quarterly Visits (Week 12, 24, 36, etc)	Beginning with Week 12, quarterly visits consist of all ~~weekly (Section 12.3.2) and~~ monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below: • PK sample • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)	15
Section 12.3.6, Interim Dosing Visit	This visit has been removed	15

Section 12.3.7, Unscheduled Hypersensitivity Reaction Visit	• Vital signs • Urine/albumin creatinine ratio • ~~Sedimentation rate~~ • Urine N-methyl histamine • ~~PK sample~~ Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects ~~who do not have an IgE-mediated reaction~~ may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into the clinic for at least 8 weeks. ~~If laboratory data shows that the subject experienced an IgE-mediated reaction, dosing will not resume until review of the case with the Investigator, Sponsor's Medical Officer and an allergist/immunologist (refer to Section 9.1.1.3.3).~~	6, 15

Section 14.2, Safety Analysis	The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). ~~All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).~~ The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug.Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented. Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics~~, including clinically significant changes from baseline,~~ of vital signs, physical examination results, ECG results, and X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.	8

Section 14.4, Efficacy Analysis	Change in blood Phe concentration from baseline (to be defined in the SAP) to each scheduled time point and presence/absence of antibodies will also be summarized. The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis. ~~Information regarding this exploratory analysis will be provided in the Statistical Analysis Plan.~~ ~~For subjects who completed the Substudy, the effect of stopping and re-starting rAvPAL-PEG dosing on safety, blood Phe level, and immune response will be explored. Information regarding the analysis~~Details regarding exploratory analyses will be provided in the SAP ~~Statistical Analysis Plan~~.	17

Section 15, Data Monitoring Committee	The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study. ~~Meetings of the DMC can be convened at any time at the discretion of the DMC Chair or the Sponsor's Medical Officer. The Chair will be notified by the Sponsor's Medical Officer of all SAEs and will receive summaries of other safety data as available. The DMC will review the study data as soon as it is available during the study, on a schedule defined in the DMC Charter, and offer advice on whether or not to proceed, modify or terminate study enrollment on the basis of toxicity. Details regarding the DMC membership and safety data to be reviewed by members will be provided in a charter.~~ The responsibilities of the DMC are to: • Assess the effect and relevance of new external evidence. • Make recommendations to the BioMarin concerning continuation or termination of the study or other study modifications based on observations.	17

Section 16, Compensation, Insurance, and Indemnity	….The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment, unless the executed Clinical Trial Agreement between BioMarin and the institution of the investigator specifies otherwise. If the subject has followed the Investigator's instructions, BioMarin ~~will~~may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures~~, if these costs are not covered by health insurance or another third party that usually pays these costs~~. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.	17
Section 17, Case Report Forms and Source Documents	BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent. ~~If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an "interview" style of data verification.~~	17

BIOMARIN®

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria (PKU)
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Monitor:	[redacted], MD [redacted], Clinical Sciences BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Up to 98 months or until study is terminated
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg or 375 mg/week
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011
Date of Amendment 4:	June 7, 2012
Date of Amendment 5:	February 28, 2014
Date of Amendment 6:	October 30, 2014

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 6

Date: October 30, 2014

RATIONALE AND SUMMARY OF CHANGES

The protocol for Study PAL-003 is being amended to make the following changes:

1. All subjects who are on stable doses of rAvPAL- PEG, including subjects on doses between 70 mg/week and 350 mg/week (inclusive) may remain in this study to continue open-label administration with rAvPAL-PEG. Subjects may be eligible for participation in the long-term, open-label extension portion of the Phase 3 study, 165-302. These revisions align with changes being made to the BMN 165 Phase 3 study, 165-302.
2. The individual and study stopping criteria have been revised and reflect a better understanding of the risk/benefit with rAvPAL-PEG administration. Stopping criteria revisions are aligned with recommendations from an independent Data Monitoring Committee (DMC) and the Food and Drug Administration (FDA).
    a. 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.
    b. 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.
    c. 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 to sponsor for review.
3. Instructions have been added for reintroducing study drug following anaphylaxis.

4. Safety precautions have been added:
    a. Premedications administered prior to study drug for subjects who have had a hypersensitivity AE have been revised based on a better understanding of the safety profile of rAvPAL-PEG. Changes are also consistent with recommendations from an independent immunologist. Should premedication be required, H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) should be administered approximately 2-3 hours prior to study drug per investigator determination. NSAIDs should be given with food and may be omitted if not tolerated.
    b. 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. Sites will assess that the epinephrine injectors have not expired and that subjects are adhering to instructions.
5. Information to track and categorize device (prefilled syringe) malfunction and failures, device-related injuries, and any medication errors attributed to device performance has been added.
6. 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. The collection and testing of serum anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM and anti-rAvPAL-PEG neutralizing antibodies for Unscheduled Hypersensitivity Visits are no longer necessary as all these antibodies are tested frequently (at routine study visits) and can provide results at or near the time of an hypersensitivity AE.
7. rAvPAL-PEG dosing information has been revised to include instructions specific to prefilled syringe. Self-administration training for prefilled syringe drug product has been added.
8. The study duration has been extended for another 12 months. The maximum study duration is now 98 months.
9. The final follow-up visit schedule has been changed from 1 week to 4 weeks after the final dose to better monitor safety by collecting AEs and SAEs during this period, and to align with the Early Termination Visit as well as other BMN 165 protocols.
10. The requirement for observed dosing in clinic following dose interruption has been extended from one day following the interruption to one week following interruption. This revision aligns the requirement for observed dosing following interruption in PAL-003 with that in the 165 phase 3 studies.
11. The injection site inspection procedure has been discontinued.

12. The criteria for subject eligibility to self-administer study drug has been revised. All subjects in PAL-003 have now been receiving home-based dosing for at least a year; in addition, all subjects in the ongoing Phase 3 BMN 165 studies are dosed at home. Safety data to date indicate that mild to moderate hypersensitivity adverse events commonly occur in subjects receiving BMN 165 but can generally be safely managed with continued home-based dosing. Safety precautions including detailed guidance regarding response to hypersensitivity AEs are being added to all ongoing studies; this guidance supersedes previous PAL-003 instruction mandating return to clinic-based dosing for all hypersensitivity reactions or Grade 3 or higher adverse events. In addition, PAL-003 experience has shown that dosing adjustments (either increase or decrease) are occasionally necessary to maintain optimal Phe control in the long term and can be safely made without return to clinic-based dosing.
13. The rAvPAL-PEG immunogenicity profile has been revised and updated. Information that characterizes the specific antibody responses detected in subjects has been added.
14. Additional minor changes have been made to improve clarity and consistency.

Specific major revisions since the completion of the last protocol amendment (Amendment 5, dated 28FEB2014) to the text of each section are outlined in Section 24.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data were collected for subjects who have already achieved Phe reduction to within the protocol-defined target range. This substudy has been completed as of 20 December 2013.

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OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

STUDY DESIGN AND PLAN:

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first dose will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Additionally, subjects will be given two epinephrine injectors and will be instructed to carry one epinephrine injector with them at all times (refer to Section 9.4). A follow-up telephone call will be made to the

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subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Additional training will be provided to subjects and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Month 98 visit.

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- The study is terminated.
- The study drug receives marketing authorization.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

PAL -003 Substudy

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed. This substudy has been completed as of 20 December 2013 .

Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.

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- The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

Safety Assessment Criteria:

Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and non-steroidal

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anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality. Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG. Dosing instructions are presented in Table 9.1.1.2.1 and are regardless of previous occurrence.

Once an AE (other than anaphylaxis) improves to grade 1 or resolves, study drug dose may be increased, maintained, or reduced. If dosing has been interrupted due to an AE (other than anaphylaxis) and the investigator determines it is safe for the subject to resume dosing, the first dose after improvement of the AE should be performed in the clinic. The subject must be observed for 30 minutes following administration due to restarting dosing. The subject may be advised to premedicate with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to subsequent BMN 165 doses. NSAIDs should be given with food and may be omitted if not tolerated.

Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access.

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The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated.

Stopping Criteria:

If an NCI-CTCAE grade ≥3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

Brown's severity criteria are presented in Table 9.1.2.1.

NUMBER OF SUBJECTS PLANNED:

Up to 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.
4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of

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childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.

5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's medical monitor. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or:

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or:

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Refer to Table 9.4.2.1.1 for information on prefilled syringe volume, concentration, and dose.

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Subjects should convert their vial and syringe dose to prefilled syringe dose using information presented in Table 9.4.2.1.2.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Fixed Dosing

Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects should convert their weight-based dose using information presented in Table 9.4.4.1.

DURATION OF TREATMENT:

Up to 98 months.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, anti-rAvPAL IgE, and anti-PEG-PAL IgE) will be assessed.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in a previous rAvPAL-PEG study may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics for vital signs, physical examination results, ECG results, X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the Statistical Analysis Plan [SAP]) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

Details regarding exploratory analyses will be provided in the SAP.

Substudy Analysis:

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

3 TABLE OF CONTENTS

LIST OF TABLES

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure

DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	identification
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies

NCI	National Cancer Institute
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NOAEL	no observable adverse effect level
NSAID	non-steroidal anti-inflammatory medication
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Pharmacovigilance Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively,

and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given ≥ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.

7.2.1 Phase 1 Study, PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.
- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002, PAL-004, and 165-205

Based on results from the Phase 1, single-dose study, the Phase 2 studies have been designed to evaluate rAvPAL-PEG at various doses and dosing regimens to safely achieve and maintain blood Phe reductions in subjects with PKU. Currently, there are three ongoing Phase 2 studies (PAL-002, PAL-004, 165-205); an overview of each study is presented in Section 7.2.2.1 (PAL-002), Section 7.2.2.2 (PAL-004), and Section 7.2.2.3 (165-205).

7.2.2.1 Study PAL-002

Study PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria) was initiated in September 2009 and has completed enrollment. As of 10 September 2012, 40 subjects have been enrolled and 37 have completed the study. PAL-002 enrolled 11 previously exposed subjects from the single-dose study, PAL-001, and 29 subjects who were naïve to rAvPAL-PEG. Thirty-three of the 37 subjects who completed PAL-002 enrolled into this open-label extension study (PAL-003). Two subjects discontinued from the study due to lost to follow-up, relocation, or other reasons, and 1 subject discontinued from the study due to an AE (skin reaction).

The primary objective of this study was to evaluate the effect of multiple doses of rAvPAL-PEG (ranging from 0.001 mg/kg/week to 1.0 mg/kg/week) on blood Phe concentrations in subjects with PKU with up to 16 weeks of treatment. The secondary objectives of the study were to evaluate the safety and tolerability of SC injections of multiple doses of rAvPAL-PEG, to evaluate the immune response to rAvPAL-PEG, and to evaluate the PK profile of rAvPAL-PEG in subjects with PKU.

The PAL-002 study design consists of two parts. In Part 1, rAvPAL-PEG was administered as a once weekly fixed, low-dose induction regimen for 8 weeks. In Part 2, the rAvPAL-PEG dose was titrated upwards; subjects received adjustable dose increases for up to 8 weeks to achieve a target blood Phe concentration of 600 µmol/L. The doses and dosing schedules were revised with a series of protocol amendments to incorporate the information gained during conduct of this early, open-label, multiple-dose study.

A wide range of doses was planned for the PAL-002 study, beginning with doses as low as 0.001mg/kg/week. However, no substantial reductions in blood Phe level were observed in the majority of subjects who were administered rAvPAL-PEG in the initial four cohorts at doses of 0.001, 0.003, 0.01, and 0.03 mg/kg administered once per week. The absence of appreciable Phe reduction after 16 weeks of treatment in this range of doses led to a decision to amend the study protocol to include a cohort with a higher starting dose of 0.1 mg/kg/week, which had been previously shown to decrease blood Phe levels to approximately 600 µmol/L in the single-dose, Phase 1 study (PAL-001). After the first 2 weeks of dosing at 0.1 mg/kg/week, transient Phe reduction was observed in this subset of subjects. However, this dosing regimen was accompanied by mild to moderate hypersensitivity reactions primarily after the second weekly dose, suggesting that additional exploration of dosing regimens would be required.

In summary, preliminary results from PAL-002 demonstrated that doses below 0.1 g/kg administered once per week were not effective in reducing Phe levels. While transient reduction of Phe was apparent at doses of at least 0.1 mg/kg given once per week, this dose regimen was associated with a high incidence of systemic hypersensitivity reactions, typically following the second weekly dose. Further escalation above this dose of 0.1 mg/kg/week would be required to sustain the effect on Phe levels over time. Increased doses would require spreading dose administration over several days per week, as once-weekly administration of higher doses would not be practical due to the large volume of study drug. Therefore, additional dosing regimens would need to be explored in a subsequent study (PAL-004).

Once subjects completed Study PAL-002, they were eligible to enroll into this open-label extension study, PAL-003 to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.2 Study PAL-004

PAL-004 (An Open-Label Study to Evaluate the Safety, Tolerability, and Efficacy of Subcutaneous Dose Levels of rAvPAL-PEG Administered Daily in Subjects With Phenylketonuria) was initiated in April 2011 and has completed enrollment. As of 10 September 2012, 16 subjects have been enrolled, 15 subjects have completed the study, and 15 subjects have enrolled into this study (PAL-003). One subject withdrew consent from continued participation prior to study completion. All subjects enrolled in this study were naïve to rAvPAL-PEG exposure.

The objective of this study was to determine if daily administration (defined as 5days/week) of rAvPAL-PEG at dose levels of 0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day was safe and effective in reducing and maintaining blood Phe concentrations to 600 µmol/L in subjects with PKU. The starting dose was 0.4 mg/kg, administered 5 days/week. A total of 12 subjects were added in sequence and were started on doses of 0.4, 0.2, 0.1, 0.06 mg, or 0.001/kg/day based on incoming safety data. Overall, subjects in PAL-004 who initiated treatment at higher daily doses of 0.1 to 0.4 mg/kg/day achieved immediate and substantial reduction of blood Phe levels, but dosing had to be temporarily reduced or interrupted due to the onset of systemic hypersensitivity reactions at approximately Day 10 of dosing. Phe reductions did not persist in the setting of temporary dose reduction or interruption, but Phe levels generally improved if higher dose levels were reinstated.

To prevent the onset of hypersensitivity reactions that were temporally associated with the onset of anti-drug IgM responses, an additional dosing strategy was assessed in PAL-004;

4 subjects initiated dosing at a low dose of 0.001mg/kg/day or 0.06mg/kg/day for 5 consecutive days and then dosing was suspended for 2 weeks. Dosing was restarted on Day 21 at the initial dose followed by dose titration to target Phe levels. Subjects administered this dosing regimen had similar efficacy, but the incidence of hypersensitivity reactions appeared to be similar to that of subjects who did not have the planned dose interruption, suggesting little advantage with this alternate induction regimen.

Experience from PAL-004 indicated that initiating daily rAvPAL-PEG dosing with relatively high daily doses was not sustainable due to the onset of hypersensitivity reactions that occurred approximately 9 to 12 days after the start of administration, nor was the alternate dosing regimen (2 week drug holiday) well tolerated or effective in Phe reduction. In addition, the daily treatment regimen did not reduce the time to achieve target blood Phe levels seen in previous studies. This study indicated that to improve tolerability, rAvPAL-PEG should be initiated with an induction period at doses substantially lower than 0.4mg/kg, followed by upward dose titration toward target Phe levels.

Once subjects completed Study PAL-004, they were eligible to enroll into this open-label extension study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.3 Study 165-205

Study BMN 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy, and Tolerability of Subcutaneously Administered rAvPAL-PEG in Subjects With PKU for 24 Weeks) was initiated in May 2012. Data from ongoing Studies PAL-002, PAL-004, and PAL-003 suggested that a 4-week treatment course with weekly dosing at a fixed low dose (induction), followed by a weekly 2-fold, upward titration of rAvPAL-PEG to approximately 10-fold higher than the initiation dose (maximum of 375 mg/week) is effective and well tolerated. The objective of this study was to further assess this dosing regimen. Two rAvPAL-PEG dosing regimens were to be explored in this study: weekly low-dose induction (2.5 mg/week for 4 to 8 weeks), followed by a period of upward dose titration towards a target Phe level (600 µmol/L) followed by maintenance dosing at that level through the 24 week study duration (Group 1). The other dosing regimen planned for this study (Group 2) involved administration of a single bolus dose of 8 mg, followed by a treatment holiday of at least 3 weeks duration followed by resumption and escalation of study drug administration following a pattern similar to that employed in Group 1. A total of 24 subjects were enrolled into 165-205. In addition to confirming an effective, well-tolerated dose regimen, this study incorporated non-weight-based dosing, starting with dose

administration once per week at a low dose (2.5mg) with gradual escalation and conversion of dose administration to 5x/week dosing for chronic maintenance therapy.

Once subjects have completed Study 165-205, they are eligible to enroll into this study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week, or into the Phase 3 study, BMN 165-302.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an

exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

This study is an extension of previous rAvPAL-PEG Phase 2 studies. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from previous rAvPAL-PEG studies will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to Polyethylene Glycol (PEG)

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Immunologic Response

The active drug substance in rAvPAL-PEG is a bacterial protein and elicits immune recognition and subsequent antibody responses. Antibody epitopes on the protein are expected to be rendered at least partially inaccessible by the extensive PEGylation on the drug (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model but does not eliminate antibody formation. Although PEGylation of rAvPAL likely reduces the antigenicity of the molecule, 100% of individuals exposed to rAvPAL-PEG develop antibodies against rAvPAL.

Drug-specific immune responses can range from clinical irrelevance to causing serious hypersensitivity AEs (such as anaphylaxis) and may reduce efficacy due to antibody-mediated drug clearance or antibody neutralization of enzymatic activity. Historically, PEG was thought to be nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.); however, repeated studies have found that subcutaneous (SC) and intravenous exposure to PEG can induce anti-PEG antibodies (Harris, 2003, Nat.Rev.Drug Discov.), (Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). However, PEG antibody responses were associated with hypersensitivity reactions to PEGylated liposomes (Judge, 2006, Mol.Ther.). Anti-PEG antibodies have also been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.).

Subjects participating in the Phase 2 clinical studies have been monitored closely for anti-drug immunogenicity (anti-rAvPAL immunoglobulin G [IgG], anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, neutralizing antibodies that inhibit PAL enzymatic activity, anti-rAvPAL IgE, and anti-rAvPAL-PEG IgE), complements, C-reactive protein (CRP), ESR, and complete chemistry. All subjects treated with rAvPAL-PEG in the Phase 2 clinical trials developed antibodies against the PAL protein. Anti-rAvPAL IgM antibodies were first detected within 2-3 weeks of treatment initiation and anti-rAvPAL IgG antibodies were detected within 2-3 months. The anti-rAvPAL IgG response generally peaked by 3-5 months and was sustained in all subjects. Neutralizing antibodies (NAb) capable of inhibiting enzymatic activity were detected in a minority of subjects 2-3 months after treatment

initiation. The timing of the NAb response suggests these antibodies may be a component of the anti-rAvPAL IgG response. It is expected that the centralized location of the PAL active site, within the tetrameric structure, makes it difficult for antibodies to bind and inhibit enzymatic activity. In addition, anti-PAL antibody binding is likely sterically hindered by the extensive PEGylation. The majority of treated subjects also developed a transient anti-PEG antibody response. The anti-PEG response was comprised of IgM and IgG isotypes. In general, anti-PEG antibodies were no longer detected after 5-6 months of treatment. This type of transient antibody response is typical of T cell-independent type 2 (TI-2) responses.

The majority of immune-mediated AEs experienced in subjects treated with rAvPAL-PEG in the Phase 2 studies are believed to be the result of antibody-mediated hypersensitivity. The primary immune mediator is thought to be IgM immune complexes, as evidenced by the timing of onset prior to IgG development and the reduced occurrence over time. IgE positive test results have not been associated with hypersensitivity in any of the subjects thus far, and the majority of subjects have been safely rechallenged with rAvPAL-PEG following reactions. IgM immune complexes are known to be efficient activators of the classical complement pathway and can lead to the accumulation of complement component anaphylatoxins (C3a, C4a, C5a) that can induce hypersensitivity symptoms. Although IgG immune complexes can also activate the classical complement pathway, they do so with less efficiency than IgM. This is due, in part, to the requirement for multiple Fc regions in close proximity to activate the complement cascade, meaning that multiple monomeric IgG Abs are required to bind, whereas only one pentameric IgM Ab is needed to bind to activate complement. Also, in the case of rAvPAL-PEG, binding of anti-PAL IgG antibodies is likely compromised due to epitope masking by the PEGylation, which may reduce IgG-mediated complement activation *in vivo*. This complex dynamic between combined isotype-specific Ab responses and epitope accessibility is thought to be a major factor dictating the need for an induction and titration dose regimen in the treatment of rAvPAL-PEG to be implemented in this study.

7.4.3 Management of Hypersensitivity Reactions

Hypersensitivity reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30 minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of hypersensitivity reactions. Because of the potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and

after the rAvPAL-PEG injection. Subject should be pre-medicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated. Subjects who qualify for self-administration of study drug are trained to recognize a serious hypersensitivity AEs and how to respond. Additionally, subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (Section 9.4). Refer to Section 9.1.1.3 for instructions regarding resumption of study drug following resolution of symptoms consistent with a clinical diagnosis of anaphylaxis per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria (Sampson, 2006, J.Allergy Clin.Immunol.).

In the event of a hypersensitivity reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of hypersensitivity reaction symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oral or IV glucocorticoids.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. An allergy and/or immunology consultation should be sought if necessary. Detailed instructions for the management of hypersensitivity reactions are provided in the Study Reference Manual.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objective is as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity was observed in a previous rAvPAL-PEG studies. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first week of dosing will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (refer to Section 9.4). A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to clinic monthly for review with the clinical study staff.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self-administration. Additional information is provided in the Subject Self-Administration Training Materials. Additional training will be provided and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 9.1.1 and Table 9.1.2. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Month 98 visit.
- The study is terminated.
- The study drug receives marketing authorization.

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2).

The Schedule of Events and PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2

Table 9.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self-administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures	Up to daily	4 weeks after final dose		4 weeks after final dose	
Informed consent	X									
Medical history, including allergy history, and demographics	X									
Physical examination[e]	X	X			X		X		X	
Vital signs[e]	X	X		X		X			X	X
Weight	X			X						
12-lead ECG	X						X		X	
Clinical laboratory tests[f]	X			X			X		X	X[g]
Complements C_3 and C_4[h]	X				X					X[g]
Sedimentation rate		X			X		X		X	
Chest x-ray	X			X (Week 48 visit only)			X		X	
Urine pregnancy test[i]	X	X		X			X		X	
Adverse events[j,k]	X	X	X	X		X	X	X	X	X
Weekly phone call to self admin participants only (to assess AEs, Inj site reactions, concomitant medications)			X					X	X	
Concomitant medications	X	X	X	X		X	X	X	X	X
Diet query	X	X		X			X		X	

Assessments and Events	Screening[a]	Treatment Period[b,c]					Final F/U Visit	Self-administration	Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)	Interim Clinician Visit (HHRN or Site)				
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures	Up to daily	4 weeks after final dose		4 weeks after final dose	
3-Day diet diary[l]				X			X		X	
Serum antibodies[m]		X			X		X		X	X
Plasma Phe and plasma tyrosine[n]	X	X		X[m]			X		X	
Urine albumin/creatinine ratio										X
Urine N-methyl histamine										X
Plasma PK sample[o]		X			X		X		X	
Administer study drug[p]		X		X		X		X		
Training (prefilled syringe)[q]				X Transition to prefilled syringe	X Every 24 weeks only					
Skin biopsy (optional; affected and not affected area)										X
Serum tryptase level[r]										X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] A separate Screening visit for this study is required only if the time between completion of the previous rAvPAL-PEG study and enrollment into PAL-003 is greater than 28 days.

[b] Monthly visits must be performed in the clinic.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Refer to Section 9.1.1 and Section 12.3.6.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis. Urine microscopy will be performed if any urinalysis results are positive for hematuria. Refer to Table 9.7.6.1.

[g] Subjects who have a National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade ≥ 3 hypersensitivity AE should be assessed for CRP, CH50, C1, C3, and C4 within 24 hours of the reaction.

[h] Complement C3 and C4 will be collected at the Screening Visit and quarterly. Additional complement testing will be performed and as needed to resolve previous abnormal test results.

[i] If positive or equivocal, perform serum pregnancy test.

[j] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm).

[k] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or the final study visit. The reporting period for nonserious AEs is from the first administration of study drug through 4 weeks after last dose or the early termination visit. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.

[m] For unscheduled hypersensitivity reaction visit, draw serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only; sampling must be performed > 8 hours after event onset and before the next dose of study drug.

[n] Samples should be drawn at least 2.5 hours after a meal. At the Investigator's discretion, blood Phe may be collected more often should more frequent monitoring of blood Phe levels be clinically warranted.

[o] Sampling should be performed predose.

[p] Dosing is up to 5.0 mg/kg/week or 375 mg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may be re-evaluated.

[q] Initial training on the use of prefilled syringe occurs at the next monthly visit and each subsequent day after transition until (in clinic or by HHRN) it has been determined the subject will switch to prefilled syringe. Once competency is documented, subjects can self-administer study drug using the prefilled syringe. Regular refresher prefilled syringe training occurs in the study clinic every 24 weeks thereafter.

[r] Mandatory for subjects who have a hypersensitivity AE. Perform within 24 hours of event onset.

Table 9.1.2: PK Substudy Dosing Regimens

Treatment Frequency	Plasma PK Sampling	Example
Subjects who are administered study drug once per week	Pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours when possible), and pre-dose of the next weekly dose	If dosed on Monday: Obtain PK sample pre-dose when possible – pre-dose Monday, Tuesday, Wednesday, Thursday, Friday, and pre-dose the following Monday. No further PK substudy draws.
Subjects who are administered study drug two or three times per week	Pre-dose and every 24 hours during the longest period between doses, when possible, and pre-dose of the next dose.	If dosed on Monday, Thursday, and Friday: Obtain PK sample every 24 hours during the longest period between doses: Pre-dose Monday, Tuesday, Wednesday, and pre-dose Thursday. No further PK substudy draws.
Subjects who are administered study drug four to seven times per week.	Pre-dose and every 12 hours during the longest period between doses, when possible, then pre-dose of the next dose.	If dosed Monday through Friday: Obtain PK sample every 12 hours during the longest period between doses (Friday to Monday): pre-dose Friday, 12 hours post-dose Friday, Saturday (24 and 36 hours post dose), Sunday (48 and 60 hours post-dose), and pre-dose the following Monday. No further PK substudy draws.

9.1.1 Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality (Section 9.1.1.3). Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

9.1.1.1 Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

9.1.1.2 Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG. Dosing instructions are presented in Table 9.1.1.2.1 and are regardless of previous occurrence:

Table 9.1.1.2.1: Dosing Instructions in Response to Hypersensitivity Adverse Events

NCI-CTCAE Grade[a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria[d]	HRV Assessment[e]
		Maintain[b]	Reduce[c]	Interrupt[c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No	X	(X) Optional	(X) Optional		Investigator discretion
3	Yes	X	(X) Optional	(X) Optional		Yes
3 and is suspected to meet Brown's criteria for severe[d]	Yes				X Consult with sponsor medical monitor	Yes
4[d]	Yes or No				X Consult with sponsor medical monitor	Yes

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, version 4.03; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a]NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b]The investigator will instruct the subject to maintain the rAvPAL-PEG dose at the time of AE onset until improvement to grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c]The rAvPAL-PEG dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE grade ≥3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor, the subject may be permanently discontinued from study drug.

[e]If the investigator determines that the NCI-CTCAE grade ≥3 hypersensitivity AE is related to administration with rAvPAL-PEG, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE [sampling must be performed >8 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, urinary N-methyl histamine, CRP, C3, C4, and tryptase).

9.1.1.3 Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated.

9.1.2 Study Stopping Criteria

If an NCI-CTCAE grade ≥ 3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

Brown's severity criteria are presented in Table 9.1.2.1.

Table 9.1.2.1: Brown's Severity Criteria

Brown's Criteria for Hypersensitivity Reactions	Definition
Mild (1) skin and subcutaneous tissue	Generalized erythema, urticaria, periorbital edema, or angioedema
Moderate (2) features suggested respiratory, cardiovascular, or gastrointestinal involvement	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness (pre-syncope), diaphoresis, chest or throat tightness, or abdominal pain
Severe (3) hypoxia or neurologic compromise	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, loss of consciousness, or incontinence

9.1.3 Dose Modifications

9.1.3.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

9.1.3.2 Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.

4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use an acceptable method of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's medical monitor. However, subjects who discontinued from study treatment early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.

8. Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study treatment should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study treatment include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified

letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in their previous rAvPAL-PEG study. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose that was administered upon completion of that study provided that there was no interruption in dosing. rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be adjusted per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects may be eligible to self-administer study drug if he or she meets the following criteria:

- The subject has no cognitive impairments that may increase the safety risk of self-administration per the assessment of the investigator.
- The subject has been approved for self-administration of study drug by the sponsor's medical monitor.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with two epinephrine injectors and has been trained on when and how to administer it. The subject will be instructed to carry one epinephrine injector with them at all times.

Qualified study site personnel will train each eligible subject on all procedures for self-administration of study drug (vial and syringe and prefilled syringe drug products) under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self-administer a dose at home. The subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 9.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials. Prefilled syringes are dispensed to subjects only after the subject (or caregiver) has completed training and competency is demonstrated in the clinic. Additional regular refresher training occurs in the clinic, every 24 weeks, and more often as needed.

Subjects who are eligible for self-administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.
- How to safely use and dispose of syringes, including prefilled syringes, used for injections of study drug.
- How to use a new syringe and vial or new prefilled syringe every time drug is administered.

- How to care for their injection site after an injection of study drug.
- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine injectors.
- Who to contact at the study site in case of an emergency.

The PI or the sponsor's medical monitor may request that self-administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplying the study drug to subjects who qualify for self-administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self-administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ± 0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Table 9.4.2.1.1: Prefilled Syringe Volume, Concentration, and Dose

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

Subjects should convert their vial and syringe dose to prefilled syringe dose using the following table:

Table 9.4.2.1.2: Conversion from Vial and Syringe Dose to Prefilled Syringe Dose

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
0 – 3.7	2.5	1	0	0
3.8 – 6.2	5	2	--	--
6.3 – 8.7	7.5	3	--	--
8.8 – 11.2	10	--	1	--
11.3 – 13.7	12.5	1	1	--
13.8 – 16.2	15	2	1	--
16.3 – 18.7	17.5	3	1	--
18.8 – 21.2	20	--	--	1
21.3 – 23.7	22.5	1	--	1
23.8 – 26.2	25	2	--	1
26.3 – 28.7	27.5	3	--	1
28.8 – 31.2	30	--	1	1
31.3 – 33.7	32.5	1	1	1

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
33.8 – 36.2	35	2	1	1
36.3 – 38.7	37.5	3	1	1
38.8 – 41.2	40	--	--	2
41.3 – 44.9	42.5	1	--	2
45 – 54.9	50	--	1	2
55 – 64.9	60	--	--	3
65 – 74.9	70	--	1	3
75 – 84.9	80	--	--	4
85 – 94.9	90	--	1	4
95 – 104.9	100	--	--	5
105 – 114.9	110	--	1	5
115 – 124.9	120	--	--	6
125 – 134.9	130	--	1	6
135 – 144.9	140	--	--	7
145 – 154.9	150	--	1	7

[a] For <45 mg/day, round to nearest 2.5 mg; for ≥45 mg/day, round to nearest 10 mg.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60 600 µmol/L), as well as any adverse events (any hypersensitivity AE) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

For vial and syringe, drug product packaging will be identified with the lot number and expiration date and will be provided in a box labeled with the study number.

For prefilled syringe, drug product packaging will be identified with the size (2.5 mg, 10 mg, or 20 mg), lot number, and kit identification (ID). The expiration date will be available in a separate Certificate of Compliance issued for each lot.

9.4.3 Storage

IP must be stored at 5 ± 3° C (41 ± 5° F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose).

For subjects receiving weight-based dosing, dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1.

Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 15 mg/ml concentration (ml) (vial)[a]
0.03	2.5	0.17
0.06	5	0.33
0.12	10	0.67
0.25	20	1.33
0.5	40	2.67
1.0	75	5
2.0	150	10
3.0	225	15
4.0	300	20
5.0	375	25

[a] Applies to both 1.0 and 1.3 ml withdrawal.

It is preferable that the injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff, other qualified and trained study personnel, or qualified subjects.

Information for dosing of subjects who enroll into this study after completing previous rAvPAL studies is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in their previous rAvPAL-PEG study.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self-administer study drug at home if approved by the sponsor's medical monitor and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the sponsor's medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product will be encouraged to be premedicate as outlined in Section 9.1.1. If the hypersensitivity AE worsens with a repeat injection (as determined by the Investigator in consultation with the sponsor's medical monitor) even with premedication prior to study drug

dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.3.2).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for dosing of subjects who enroll into this study after completing previous rAvPAL-PEG studies is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator. After a dosing interruption of ≥ four consecutive doses, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first week of dosing of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30 minutes following administration due to restarting dosing. Premedication with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to study drug should be administered following any dose interruption of ≥ four consecutive doses. NSAIDs should be given with food and may be omitted if not tolerated. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1) during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study treatment per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or

designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self-administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next monthly study visit. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a] or CRO	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Pregnancy test (urine) and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1
Urinalysis	Central laboratory	9.7.6

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL IgE and anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant

abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn monthly, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1).

Blood Phe may be collected more often than monthly at the discretion of the Investigator should more frequent monitoring of blood Phe levels be clinically required.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed. For subjects participating in the Substudy, PK sampling will be performed as follows:

- For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.
- For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose.
- For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

See Table 9.1.2 for additional details.

BioMarin will perform the analysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods

consumed for 3 consecutive days each month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.4 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1).

BioMarin or CRO will perform all anti-body testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Phenylalanine
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Sedimentation rate[a]
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Spot urine albumin/creatinine ratio	**Additional Unscheduled Hypersensitivity Reaction Visit Tests[b]**
Total protein		Nitrite	CH50
Calcium		Urobilinogen	C_1, C_3, C_4
Sodium		Hemoglobin	Serum tryptase level[b]
Potassium		Bilirubin	CRP
Glucose			Urine N-methyl histamine
			Urine albumin/creatinine ratio
Uric acid			**Complement Testing[c]**
CO_2			C_3
Chloride			C_4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory.

[b] Perform within 24 hours of event onset.

[c] Complement C_3 and C_4 to be drawn at the Screening Visit and then quarterly or as needed to resolve abnormal test results.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study AE reporting period is as follows: After informed consent but prior to initiation of study treatment, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, all non-serious AEs and SAEs reporting period begins and continues until 4 weeks following the last administration of study drug or the early termination visit, whichever is longer (refer to Section 12). The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

For this study, a medical device is defined as the prefilled syringe and all of the component parts. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe. Events assessed by the investigator as related to the device, including malfunction, injury, or medication error, will be captured into an electronic data capture system. All serious events related to the device will be reported to BioMarin Pharmacovigilance (BPV) within 24 hours using the device-related event report form and entered onto the appropriate eCRF page(s) as required.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or study participation is not the cause of the AE. Outcome of AEs (and resolution dates) should be documented on the appropriate CRF page(s) and in the subject's medical record unless the subject is lost to follow-up or it has been determined that the study treatment or study participation is not the cause of the AE.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)[b]
4	Life threatening or debilitating: consequences; urgent intervention indicated
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	*Description*
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
    - Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Important medical events or reactions that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes above should also usually be considered serious.

For this protocol, anaphylaxis per NIAID/FAAN criteria for the clinical diagnosis of anaphylaxis is designated as an AE of special interest (serious or nonserious and irrespective of severity) to facilitate rapid reporting and sponsor review. All occurrences of anaphylaxis per NIAID/FAAN criteria will be reported to the sponsor within 24 hours of the site becoming aware of the event using the SAE form. Severity and serious criteria (if applicable) should be reported on the SAE form.

The reporting period for SAEs related to protocol imposed interventions begins after informed consent is obtained, the reporting period for all SAEs begins after first dose of study drug and continues until 4 weeks following the last administration of study drug or End of Treatment Visit. The reporting period for Serious Device Related Events begins after first dose of study drug and ends with the last administered dose.

All SAEs (including serious device-related events), whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form or device Report Form to BioMarin Pharmacovigilance (BPV). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study treatment or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the subject's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the End of Study CRF. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution to BPV in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (i.e., modified dose amount or frequency not defined in protocol).

- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.5 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the Sponsor's Medical Office sponsor's medical monitor r any AEs for which the issue of seriousness is unclear or questioned. Contact information for the sponsor's medical monitor is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to clinic on a monthly basis for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis)
- Complement C_3 and C_4
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. All study activities will occur on the first day of each week unless otherwise noted. On days that a dose is given, assessments will be done predose unless

otherwise specified. Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator)

12.3.2 Weekly Telephone Visit

Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:

- Injection-site self inspection (previous and current injection site)
- Assessment of AEs
- Concomitant medications

- Confirmation of dose level, frequency, and study drug administration

12.3.3 Substudy Visits

For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2.

12.3.4 Monthly Visits (Week 4, 8, 12, etc)

Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 4, monthly visits must be performed in the clinic. The following study activities will be performed:

- Vital signs
- Weight
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray.
- Chest x-ray (Week 48 visit only)
- Assessment of AEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.)
- Prefilled syringe self-administration training (to be performed prior to subjects transitioning from vial and syringe drug product to prefilled syringe drug product).
- If additional training is needed on days subsequent to the monthly visit, additional clinic visit(s) or HHRN may be used.

12.3.5 Quarterly Visits (Week 12, 24, 36, etc)

Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 12, quarterly visits consist of all monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below:

- Physical examination
- Complement C_3 and C_4
- Sedimentation rate
- PK sample
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Prefilled syringe refresher training (every 24 weeks only)

12.3.6 Unscheduled Hypersensitivity Reaction Visit

Subjects who have an NCI-CTCAE grade ≥ 3 hypersensitivity reaction after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 24 hours) the reaction.

- Vital signs
- Clinical laboratory tests
- Urine/albumin creatinine ratio
- CRP, CH50, C1, C3, and C4
- Urine N-methyl histamine
- Serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only (performed > 8 hours after event onset)
- Serum tryptase level
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of the reaction)
- Assessment of AEs
- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects may resume study drug administration per the discretion of the Principal Investigator and the sponsor's medical monitor.

12.4 Early Termination Visit

The Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug. Subjects who terminate from study treatment early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

12.5 Final Follow-up Visit

The final follow-up (F/U) Visit will occur 4 weeks after the final dose of study drug.

The following study activities will be performed at the F/U Visit:

- Physical examination
- Vital signs, including weight
- 12-lead ECG
- Clinical laboratory tests

- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Final Follow-up Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF).

The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics of vital signs, physical examination results, ECG results, and X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should

data become available from previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

14.3.1 Pharmacokinetic Substudy Analysis

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored. This substudy has been completed as of 20 December 2013.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the SAP) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis.

Details regarding exploratory analyses will be provided in the Statistical Analysis Plan.

14.5 Determination of Sample Size

Subjects who participated in previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Assess the effect and relevance of new external evidence.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.2.
- Make recommendations to the BioMarin concerning continuation or termination of the study or other study modifications based on observations.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment, unless the executed Clinical Trial Agreement between BioMarin and the institution of the investigator specifies otherwise. If the subject has followed the Investigator's instructions, BioMarin may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between BioMarin and the PI.

21 REFERENCES

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003, Amendment 6

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

[redacted]

30 OCT 2014

Signature Date

Printed name: [redacted]

24 PROTOCOL AMENDMENT MAJOR TEXT REVISIONS

Section no./Title	Text Revisions
	Rationale #1: **Eligibility for receiving open-label drug in current or extension study**
9.1 Overall Study Design and Plan	~~Subjects who participate in this study will be required to transition into a Phase 3 study (165-302) if weekly doses of rAvPAL-PEG are between 70 mg/week and 350 mg/week (inclusive) provided that doses of rAvPAL-PEG have been stable (ie, no major change in dose for at least 4 weeks). Subjects who meet these criteria must be transitioned from PAL-003 to Study 165-302 provided they meet the 165-302 study eligibility criteria.~~ ~~Subjects who are eligible for Study 165-302 but do not transition into 165-302 will be withdrawn from this study.~~ ~~The PAL-003 study will continue until BMN-165 is approved by the FDA for marketing in the United States or until the sponsor terminates further development of this drug for treatment of PKU.~~
	Rationale #2a-c: **Revisions to Stopping Criteria, AEs of special interest**
9.1.1.1 Individual Stopping Criteria	**9.1.1.1 Individual Stopping Criteria** Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.
9.1.1.2 Dosing in Response to Hypersensitivity Adverse Events	**9.1.1.2 Dosing in Response to Hypersensitivity Adverse Events** Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG. Dosing instructions are presented in Table 9.1.1.2.1 and are regardless of previous occurrence: **PLEASE SEE TABLE 9.1.1.2.1 AT THE END OF SECTION 24**
	9.1.2 Study Stopping Criteria If an NCI-CTCAE grade ≥ 3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening

	or required treatment to prevent a life-threatening event: • Cyanosis or $SpO_2 \leq 92\%$ • Hypotension with SBP < 90 mm Hg (adults) • Neurologic alteration: loss of consciousness, collapse, incontinence Brown's severity criteria are presented in Table 9.1.2.1. **PLEASE SEE TABLE 9.1.2.1 AT THE END OF SECTION 24**
9.1.3 Safety Assessment Criteria	~~**9.1.3 Safety Assessment Criteria**~~ ~~If an individual subject exhibits toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, that subject will have dosing halted until the toxicity resolves. The subject's data will be evaluated by the Principal Investigator (PI) and the Sponsor's Medical Officer, and a decision will then be made whether to discontinue the subject from the study treatment, restart dosing at the same dose level, or restart dosing at a lower dose level.~~
9.1.4 Stopping Criteria	~~**9.1.4 Stopping Criteria**~~ ~~If 2 or more subjects at a dose level exhibit toxicity of a treatment-emergent CTCAE grade 3 or greater or a systemic reaction that is assessed as related to treatment with study drug, further dosing of subjects at that dose level will evaluated and a safety assessment will be completed. In addition, the Food and Drug Administration (FDA) may be notified of this occurrence if appropriate.~~
10.2 Serious Adverse Events	For this protocol, anaphylaxis per NIAID/FAAN criteria for the clinical diagnosis of anaphylaxis is designated as an AE of special interest (serious or nonserious and irrespective of severity) to facilitate rapid reporting and sponsor review. All occurrences of anaphylaxis per NIAID/FAAN criteria will be reported to the sponsor within 24 hours of the site becoming aware of the event using the SAE form. Severity and serious criteria (if applicable) should be reported on the SAE form.
	Rationale #3: **Resumption of drug after anaphylaxis**
9.1.1.3 Response to Anaphylaxis	**9.1.1.3 Response to Anaphylaxis** If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and

	anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level. For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated.
	Rationale # 4a, 4b: **Safety precautions added regarding premedication and epinephrine injectors**
7.4.3 Management of Hypersensitivity Reactions	…Because of the ~~small~~ potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection. Subject should be pre-medicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated. Subjects who qualify for self-administration of study drug ~~will be provided with emergency resuscitation~~ are trained to recognize a serious hypersensitivity AEs and how to respond. Additionally, subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (Section 9.4). Refer to Section 9.1.1.3 for instructions ~~(refer to Section 9.4 and the Subject Self-Administration Training Materials).~~regarding resumption of study drug following resolution of symptoms consistent with a clinical diagnosis of anaphylaxis per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria (Sampson, H. A., et al, 2006, J Allergy Clin Immunol). • Administration of additional symptomatic treatment ~~(eg, acetaminiophen or ibuprofen)~~. … An allergy and/or immunology consultation should be sought if necessary. ~~Information regarding the management of local skin, large local skin, and systemic reactions is provided in Section 9.11.~~ Detailed instructions…
9.1.1 Response to Hypersensitivity Adverse Events	~~**9.1.1 Management of Local and Systemic Reactions to rAvPAL-PEG**~~ ~~Subjects who qualify for self-administration of study drug will be provided with information and instruction with regard to management of local and systemic reactions (refer to the Subject Self-Administration Training Materials).~~ **9.1.1 Response to Hypersensitivity Adverse Events** Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis)

and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality (Section 9.1.1.3). Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

~~**9.1.1.1 Subjects Who Have Had Previous Hypersensitivity Reactions to rAvPAL-PEG or a PEG-containing Product**~~

~~Subjects who had a previous systemic reaction to rAvPAL-PEG or a PEG-containing product that warranted early termination from a previous rAvPAL-PEG study are excluded from participation in this study. Subjects who have had a previous local skin reaction to rAvPAL-PEG in a previous rAvPAL-PEG study are eligible to participate in this study. Subjects who have had a previous reaction and are deemed eligible for participation must be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing for the remainder of the study. The premedication dosage will be standard. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable). Subjects may develop systemic, large local skin, or local skin reactions after enrollment in PAL-003. Refer to Section 9.1.1.2 for definitions of systemic and local skin reactions. For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and 9.1.1.3.1.~~

~~**9.1.1.2 Definition of Reaction and Hypersensitivity Reactions to rAvPAL-PEG Administered Subcutaneously**~~

~~During Study PAL-003, subjects may experience hypersensitivity reactions to rAvPAL-PEG. The hypersensitivity reactions are defined as follows:~~

~~Local skin reaction:~~

- ~~Skin signs or symptoms in 1 affected primary location, ie, hives, wheals, or swelling or an area of erythema, redness, induration, pain, or itching at or near the site of injection.~~

~~Large local skin reaction:~~

- ~~Skin signs or symptoms, ie, hives, wheals, or swelling or an area of erythema, redness, or induration with pain or itching that extends beyond the site of injection by approximately 6 inches (15 cm) and/or signs or symptoms that are not contiguous to the injection site. These may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure and, therefore, will be managed as a systemic reaction when not contiguous to the injection site.~~

~~Systemic reaction (including generalized skin symptoms):~~

- ~~Skin and non-skin signs or symptoms in more than 1 affected primary location, ie, cutaneous reaction in more than 1 area and/or anaphylaxis or any other generalized symptoms, such as hypotension, angioedema or the involvement of other organ systems, eg, respiratory, cardiovascular, gastrointestinal, neurological; and/or a fever attributed to treatment with rAvPAL-PEG (≥ 100.4°F or ≥ 38°C).~~

~~For management of hypersensitivity reactions that occur during this study, refer to Section 9.1.1.3 and Figure 9.1.1.3.1.~~

[redacted]

[redacted]

[redacted]

[redacted]

~~9.1.1.3.1 Local Skin Reactions~~

~~All subjects who experience a local skin reaction during this study may be premedicated orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Recommended premedications are acetaminophen and/or non-sedating antihistamine. The dosage will be standard. Subjects must be accompanied by a designated driver (if applicable) because antihistamines can cause drowsiness. Symptoms of local reactions may be treated with local application of ice and non-sedating oral and/or topical antihistamines and/or topical steroids (refer to Section 7.4.3).~~

~~If the local skin reaction worsens with a repeat injection (as determined by the Investigator in consultation with the Sponsor's Medical Officer) even with premedication prior to study drug dosing, the subject must be withdrawn from study treatment.~~

~~9.1.1.3.2 Large Local Skin Reactions~~

~~Large local skin reactions (refer to Section 9.1.1.2 for a definition) may or may not be contiguous to the rAvPAL-PEG injection site, and subjects with either type of large local skin reaction may continue dosing if the skin symptoms have resolved and no other symptoms have developed. Subjects will be encouraged to premedicate orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses.~~

~~Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.3).~~

~~9.1.1.3.2.1 Large Local Skin Reactions Contiguous to Injection Site~~

~~Subjects who develop a large local skin reaction contiguous to the injection site after administration of rAvPAL-PEG or to rAvPAL-PEG or a PEG-containing product may remain in the study if the skin symptoms have resolved and no other symptoms have developed. For the remainder of the study, subjects will be encouraged to premedicate orally with acetaminophen and/or non-sedating antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Because antihistamines can cause drowsiness, it may be administered only if the subject is accompanied by a designated driver (if applicable).~~

~~Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.3).~~

~~9.1.1.3.2.2 Large Local Skin Reactions Not Contiguous to Injection Site~~

~~Large local skin reactions that are not contiguous to the injection site may be associated with a low risk of systemic symptoms or anaphylaxis upon re-exposure to rAvPAL-PEG and, therefore, will be managed as a systemic reaction (refer to Section 9.1.1.3.3). An example of a large local reaction that is not contiguous to the injection site is if an injection took place in the right upper arm and there are lesions beyond the elbow joint in the right forearm or lesions beyond the shoulder joint on the right trunk.~~

~~Symptoms of large local skin reactions may be treated with local application of ice and topical, oral, or IV antihistamines and/or steroids as needed (refer to Section 7.4.3).~~

~~9.1.1.3.3 Systemic Reactions~~

~~Subjects who experience a systemic reaction (refer to Section 9.1.1.2 for a definition) after administration of rAvPAL-PEG must stop further administrations of rAvPAL-PEG and must immediately return to the clinic for safety assessments. Subjects who experience generalized skin symptoms are included in this category. Immediately after the systemic reaction (within 24 hours), subjects with generalized skin symptoms must have additional assessments performed for safety (ie, Unscheduled Hypersensitivity Reaction Visit): serum antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG IgE, anti-rAvPAL-PEG neutralizing antibodies); serum tryptase level (it is recommended that this sample be drawn immediately after reaction); urine albumin/creatinine ratio; urine N-methyl histamine; CRP, CH50, C_1, C_3, and C_4; skin biopsy (optional; affected and not affected area; to be performed within 1 week of reaction); and clinical laboratory tests (urinalysis, chemistry, hematology; refer to Figure 9.1.1.3.1.~~

~~Following the reaction, subjects will be required to complete the assessments of the Unscheduled Hypersensitivity Reaction visit, including assessment of IgE. If a subject presents with a clinical diagnosis of anaphylaxis, further dosing will be held while laboratory evaluation of IgE, as part of the Unscheduled Systemic Reaction Visit, is performed. Subjects may resume study drug administration per the discretion of the Principal Investigator and the Sponsor's Medical Officer; however, if subject was dosing at home, they will be required to come into~~

	~~the clinic for at least 1 week.~~ ~~rAvPAL-PEG administration will resume at the next lowest dose level. Restarting of rAvPAL-PEG administration following a systemic reaction must be performed in the clinic for the first week of resumed dosing. Subjects will be encouraged to premedicate orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30 minutes following every administration of study drug. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).~~ ~~If blood Phe concentrations are not 60-600 µmol/L following 1 week of daily administration at the lowered dose cohort and there is no further evidence of toxicity (ie, another systemic reaction attributed to rAvPAL-PEG administration or clinically significant abnormal laboratory test results), the subject may be administered the next highest dose level in the clinic per the allowable dose increases outlined in Section **8.1.2**. Subjects will be encouraged to premedicate orally with acetaminophen and/or antihistamines 1-2 hours prior to study drug dosing (subjects may take non-sedating premedications prior to coming to clinic) for all future study doses. Additionally, subjects must be closely monitored for 30 minutes following every study drug administration. Because antihistamines may cause drowsiness, the subject must be accompanied by a designated driver (if applicable).~~
9.4.10 Dose Interruption and Missed Doses	During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator. ~~Subjects who miss more than 2 consecutive weekly doses (weekly dosing schedule) or 3 consecutive daily doses (daily dosing schedule) for whatever reason may continue participation in this study. After a dosing interruption~~ After a dosing interruption of ≥ four consecutive doses, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first dose of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30 minutes following administration due to restarting dosing. Premedication with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to study drug should be administered following any dose interruption of ≥ four consecutive doses. NSAIDs should be given with food and may be omitted if not tolerated. A follow-up telephone call…
9.1 Overall Study Design and Plan	Subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (refer to Section 9.4).
9.4 Treatments	The subject has been provided with two epinephrine injectors and has been trained on when and how to administer it. The subject will be instructed to carry one epinephrine injector with them at all times.
	Rationale #5: **Information tracking system for device malfunction**

10.1 Adverse Events	For this study, a medical device is defined as the prefilled syringe and all of the component parts. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe. Events assessed by the investigator as related to the device, including malfunction, injury, or medication error, will be captured into an electronic data capture system. All serious events related to the device will be reported to BioMarin Pharmacovigilance (BPV) within 24 hours using the device-related event report form and entered onto the appropriate eCRF page(s) as required.
	Rationale #6: **Changes to antibody assessment samples at Hypersensitivity Reaction Visit**
Table 9.1.1 footnote m	For unscheduled hypersensitivity reaction visit, draw serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only; sampling must be performed > 8 hours after event onset and before the next dose of study drug.
9.1.1.3 Response to Anaphylaxis	… Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug)…
12.3.6 Unscheduled Hypersensitivity Visit	Serum anti-rAvPAL ~~PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-~~ IgE and anti-rAvPAL-PEG IgE~~, and anti-rAvPAL-PEG neutralizing antibodies~~ only (performed > 8 hours after event onset)
	Rationale #7: **Prefilled syringe instructions and training**
9.1 Overall Study Design and Plan	… Additional information is provided in the Subject Self-Administration Training Materials. Additional training will be provided and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.
Table 9.1.1 footnote q	Initial training on the use of prefilled syringe occurs at the next monthly visit and each subsequent day after transition until (in clinic or by HHRN) it has been determined the subject will switch to prefilled syringe. Once competency is documented, subjects can self-administer study drug using the prefilled syringe. Regular refresher prefilled syringe training occurs in the study clinic every 24 weeks thereafter.
9.4 Treatments	Prefilled syringes are dispensed to subjects only after the subject (or caregiver) has completed training and competency is demonstrated in the clinic. Additional regular refresher training occurs in the clinic, every 24 weeks, and more often as needed.
9.4.2.1 Product Characteristics and Labeling	Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

	Subjects should convert their vial and syringe dose to prefilled syringe dose using the following table: **PLEASE SEE THE DOSE CONVERSION TABLE AT THE END OF SECTION 24**
9.4.2.1 Product Characteristics and Labeling	~~Drug~~ rAvPAL PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60 600 µmol/L), as well as any adverse events (any hypersensitivity AE) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60 -600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses. For vial and syringe, drug product packaging will be identified with the lot number and expiration date and will be provided in a box labeled with the study number. For prefilled syringe, drug product packaging will be identified with the size (2.5 mg, 10 mg, or 20 mg), lot number, and kit identification (ID). The expiration date will be available in a separate Certificate of Compliance issued for each lot.
12.3.4 Monthly Visits	• Prefilled syringe self-administration training (to be performed prior to subjects transitioning from vial and syringe drug product to prefilled syringe drug product). If additional training is needed on days subsequent to the monthly visit, additional clinic visit(s) or HHRN may be used.
	Rationale #8: **Study duration extended**
Section 9.1 Overall Study Design and Plan	The subject has completed the study through the Month ~~86~~ 98 visit.
	Rationale #9: **Change to follow-up visit schedule**
Table 9.1.1 Schedule of Events 12.5 Final Follow-up Visit	Final F/U Visit: **~~1 week~~ 4 weeks after final dose**
	Rationale #10: **Revision to length of observed dosing after dose interruption**

Section 9.1 Overall Study Design and Plan Section 9.4.10 Dose Interruption and Missed Doses	…The first ~~dose~~ week of dosing will be administered in the clinic… …The first ~~dose~~ week of dosing of rAvPAL- PEG following a dose interruption should be administered in a clinic setting…
	Rationale #11: **Inspection of injection site procedure deleted**
Table 9.1.1 Schedule of Events and footnote	Table: ~~Injection site inspection~~ Footnote: ~~Subjects must have AEs and concomitant medications assessed and an injection site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.~~
12.3 Treatment period 12.3.4 Monthly visits (Week 4, 8, 12, etc) 12.3.5 Quarterly Visits (Week 12, 24, 36, etc)	Subjects must have AEs and concomitant medications assessed ~~and an injection site inspection performed~~ whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.
12.3.4 Monthly visits (Week 4, 8, 12, etc)	~~Injection site inspection (previous and current injection site)~~
12.3.1 Day 1 (Week 1) 12.3.6 Unscheduled Hypersensitivity Reaction Visit	• ~~Injection site inspection (postdose)~~ ~~Injection site inspection~~
12.4 Early Termination Visit 12.5 Final Follow-up Visit	~~Injection site inspection (previous injection site)~~
Table 9.1.1 Schedule of	Table: ~~Injection site inspection~~

Events and footnote	Footnote: ~~Subjects must have AEs and concomitant medications assessed and an injection site inspection performed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.~~
	Rationale #12: **The criteria for subject eligibility to self-administer study drug has been revised**
9.4 Treatments	~~• The subject is on a stable dosing regimen for 2 weeks (ie, the subject has demonstrated a blood Phe level within 60-600 µmol/L).~~ ~~• The subject has not experienced any CTCAE Grade 3 or higher adverse event.~~ ~~The subject has not experienced any hypersensitivity reaction to rAvPAL-PEG for at least 4 weeks.~~
	Rationale #13: **Revision and update to the rAvPAL-PEG immunogenicity profile; additional information characterizing specific antibody responses detected in subjects**
7.4.2 Immunologic Response	**7.4.2 ~~Toxicity Due to an~~ Immunologic ~~Reaction~~ Response** The active drug substance in rAvPAL-PEG is a bacterial protein~~,~~ and ~~as such, it is expected that it may elicit~~elicits immune recognition and subsequent antibody responses. ~~Epitopes that play a role in~~Antibody epitopes on the ~~immune response may~~protein are expected to be rendered at least partially inaccessible by ~~PEG~~the extensive PEGylation on the drug (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model~~, although PEGylation~~but does not eliminate antibody formation. Although PEGylation of rAvPAL ~~may have a significant effect on reducing~~likely reduces the ~~immunogenicity~~antigenicity of the molecule, ~~it is reasonable to expect that some~~ 100% of individuals ~~may exhibit~~ exposed to rAvPAL-PEG develop antibodies against rAvPAL. Drug-specific immune responses ~~upon exposure~~ can range from clinical irrelevance to ~~the~~ causing serious hypersensitivity AEs (such as anaphylaxis) and may reduce efficacy due to antibody-mediated drug~~. These immune responses may be clinically irrelevant, cause symptoms of various degrees,~~ clearance or ~~lead to~~ antibody neutralization of ~~biological~~ enzymatic activity~~. Subjects participating in clinical trials will be monitored closely with specific antibody titers sedimentation rates, and complete blood counts (CBCs).~~ ~~As of May 2012, a total of 70 subjects have been administered rAvPAL-PEG in the single-dose, Phase 1 study (PAL-001) and in the repeat-dosing Phase 2 studies (PAL-002, PAL-004, and PAL-003). The dose levels in the repeat-dosing studies range from 0.001 to 5.0 mg/kg/week administered in various frequencies (including 5 days a week). The most clinically significant AEs have been hypersensitivity reactions that have led to dosing interruptions and reductions. Most of the hypersensitivity reactions have been nonserious and mild-to-moderate in severity.~~

	~~A total of 4 SAEs have been reported in the previous rAvPAL-PEG studies. Of these 4 SAEs, 3 have been reported as related to rAvPAL-PEG. Two of the study drug related SAEs occurred in Study PAL-001. Both of these SAEs occurred in subjects in the lower dose cohorts: an SAE of hypersensitivity reaction in a subject in the 0.001 mg/kg cohort and an SAE of anaphylactic reaction (urticaria) in a subject in the 0.01 mg/kg cohort. Neither SAE was severe or resulted in discontinuation from the study, and both of the SAEs resolved. There were no SAEs in subjects in the higher dose cohorts (0.03 mg/kg and 0.1mg/kg) in PAL-001. The third study drug-related SAE (angioedema) occurred in a subject in Study PAL-004. The fourth SAE (urticaria, dehydration) was reported as not related to study drug and occurred in a subject in Study PAL-002. There have been no reports of anaphylaxis for any subject treated with rAvPEG-PAL (as of January 2012).~~ ~~Subjects who have a systemic clinical reaction at any time during this study may undergo a series of assessments to monitor safety, including assessment of complements, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and IgE antibodies. Reintroduction of rAvPAL-PEG dosing after a reaction will depend on clinical assessments and available laboratory data, such as chemistry and urine studies, to ensure that there has been no end-organ damage prior to resuming treatment with rAvPAL-PEG. As of May 2012, antibody titers, including positive IgE titers, have not been predictive of future clinical reactions when dosing with rAvPAL-PEG. Additionally, subjects may be premedicated to prevent or mitigate risk of hypersensitivity reactions (refer to Section 9.1.1.3.3).~~ ~~PEG itself is considered nonimmunogenic Historically, PEG was thought to be nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.); however, repeated studies have found that subcutaneous (SC) and intravenous exposure to PEG can induce anti-PEG antibodies (Harris, 2003, Nat.Rev.Drug Discov.) however, antibodies against PEG may form when PEG is bound to compounds. (Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.) . In some instances, development of such antibodies did not result in any significant clinical effects in humans . However, PEG antibody responses were associated with hypersensitivity reactions to PEGylated liposomes (Judge, 2006, Mol.Ther.).In some individuals, anti-PEG antibodies may be pre-existing due to previous exposure to PEG in other products. Anti-PEG antibodies have also been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.) . Administration of rAvPAL-PEG may lead to anti-PEG antibody formation, and the duration of this possible effect is not known. Subjects in the Phase 2 rAvPAL-PEG studies have developed both anti-PEG IgM and anti-PEG IgG antibodies. Antibody formation may limit a subject's future ability to be treated with other PEG-containing (eg, Depo-Provera) or PEGylated (eg, PEG-interferon) injectable drugs. It is therefore recommended that any PEG-containing injectable drug administered to subjects after completion of this study be done under medical observation.~~ ~~**Systemic Skin Reactions**~~ ~~Two out of 25 subjects who were enrolled in Study PAL-001 and received the protocol-defined single dose of rAvPAL-PEG reported systemic skin reactions (generalized skin rash) that were attributed to treatment with study drug. One subject received a single dose of 0.001 mg/kg rAvPAL-PEG, and the other subject received a single dose of 0.01 mg/kg rAvPAL-PEG. One of these events was reported as serious, and the other was reported as nonserious; both events were reported following administration of Depo-Provera injections (medroxyprogesterone injection), an injectable contraception. These allergic reactions are thought to be related to the PEG molecules that are found in both the study drug, rAvPAL-~~

~~PEG, and Depo-Provera. These reactions are not likely to be experienced concurrent with other birth control medications.~~

~~The 2 subjects were administered study drug during PAL-001 and then experienced an allergic reaction following an injection of Depo-Provera. The symptoms of the reactions were hives on the arms and legs and difficulty breathing. The symptoms for both subjects resolved within 1 day following treatment with medication, including the antihistamine Benadryl (diphenhydramine). Both subjects were examined by an allergy specialist after they completed participation in PAL-001. A skin prick test with Depo-Provera was administered as part of this examination. One subject had a mild allergic reaction to the test; however, no further reactions or complications were reported following subsequent administration of a full dose of Depo-Provera. Upon study completion, the subject continued to receive regular doses (every 3 months) of Depo-Provera, and no further reactions were reported. The second subject also had a positive reaction to Depo-Provera following administration of the skin prick test. When a subsequent full dose of Depo-Provera was administered, [redacted] developed hives, itching, and chills. The subject's symptoms resolved within 1 day of taking medication prescribed by an allergist. See Section 7.2.1 for the results from Study PAL-001.~~

~~The active drug substance in rAvPAL-PEG is a bacterial protein that may elicit immune responses, such as urticaria, bronchospasm, itching, anaphylaxis, and others. The results from nonclinical studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys indicated the formation of anti-rAvPAL antibody titers that did not correspond with observations of injection-site reactions. Quantitation of anti-PEG titers were not assessed in the chronic repeat-dose studies in the rat and monkey. Although results from nonclincal studies of rAvPAL-PEG conducted in rats and cynomolgus monkeys did not indicate signs of immune-mediated toxicities, it is possible that the physio-chemical properties of rAvPAL-PEG may induce immunologic sensitization to the protein rAvPAL and to PEG in humans. It is currently unknown whether administration of PEG-containing drugs, such as Depo-Provera, has a sensitizing effect to PEG in rAvPAL-PEG when administered in humans. Because the exact basis for these reported events and their duration is not known, the use of PEG-containing injectable drugs prior to, during, and after this study is prohibited as a precautionary measure (refer to Section 9.3.2 and Section 9.4.8).~~

Subjects participating in the Phase 2 clinical studies have been monitored closely for anti-drug immunogenicity (anti-rAvPAL immunoglobulin G [IgG], anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, neutralizing antibodies that inhibit PAL enzymatic activity, anti-rAvPAL IgE, and anti-rAvPAL-PEG IgE), complements, C-reactive protein (CRP), ESR, and complete chemistry. All subjects treated with rAvPAL-PEG in the Phase 2 clinical trials developed antibodies against the PAL protein. Anti-rAvPAL IgM antibodies were first detected within 2-3 weeks of treatment initiation and anti-rAvPAL IgG antibodies were detected within 2-3 months. The anti-rAvPAL IgG response generally peaked by 3-5 months and was sustained in all subjects. Neutralizing antibodies (NAb) capable of inhibiting enzymatic activity were detected in a minority of subjects 2-3 months after treatment initiation. The timing of the NAb response suggests these antibodies may be a component of the anti-rAvPAL IgG response. It is expected that the centralized location of the PAL active site, within the tetrameric structure, makes it difficult for antibodies to bind and inhibit enzymatic activity. In addition, anti-PAL antibody binding is likely sterically hindered by the extensive PEGylation. The majority of treated subjects also developed a transient anti-PEG antibody response. The anti-PEG response was comprised of IgM and IgG isotypes. In general, anti-PEG antibodies were no longer detected after 5-6 months of treatment. This type of transient antibody response is typical of T cell-independent type 2 (TI-2) responses.

	The majority of immune-mediated AEs experienced in subjects treated with rAvPAL-PEG in the Phase 2 studies are believed to be the result of antibody-mediated hypersensitivity. The primary immune mediator is thought to be IgM immune complexes, as evidenced by the timing of onset prior to IgG development and the reduced occurrence over time. IgE positive test results have not been associated with hypersensitivity in any of the subjects thus far, and the majority of subjects have been safely rechallenged with rAvPAL-PEG following reactions. IgM immune complexes are known to be efficient activators of the classical complement pathway and can lead to the accumulation of complement component anaphylatoxins (C3a, C4a, C5a) that can induce hypersensitivity symptoms. Although IgG immune complexes can also activate the classical complement pathway, they do so with less efficiency than IgM. This is due, in part, to the requirement for multiple Fc regions in close proximity to activate the complement cascade, meaning that multiple monomeric IgG Abs are required to bind, whereas only one pentameric IgM Ab is needed to bind to activate complement. Also, in the case of rAvPAL-PEG, binding of anti-PAL IgG antibodies is likely compromised due to epitope masking by the PEGylation, which may reduce IgG-mediated complement activation *in vivo*. This complex dynamic between combined isotype-specific Ab responses and epitope accessibility is thought to be a major factor dictating the need for an induction and titration dose regimen in the treatment of rAvPAL-PEG to be implemented in this study.

Table 9.1.1.2.1: Dosing Instructions in Response to Hypersensitivity Adverse Events

NCI-CTCAE Grade[a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria[d]	HRV Assessment[e]
		Maintain[b]	Reduce[c]	Interrupt[c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No	X	(X) Optional	(X) Optional		Investigator discretion
3	Yes	X	(X) Optional	(X) Optional		Yes
3 and is suspected to meet Brown's criteria for severe[d]	Yes				X Consult with sponsor medical monitor	Yes
4[d]	Yes or No				X Consult with sponsor medical monitor	Yes

[a] AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, version 4.03; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[b] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[c] The investigator will instruct the subject to maintain the rAvPAL-PEG dose at the time of AE onset until improvement to grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[d] The rAvPAL-PEG dose may be reduced or interrupted if necessary per investigator determination.

[e] If a subject has an NCI-CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor, the subject may be permanently discontinued from study drug.

[f] If the investigator determines that the NCI-CTCAE grade ≥ 3 hypersensitivity AE is related to administration with rAvPAL-PEG, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE [sampling must be performed >8 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, urinary N-methyl histamine, CRP, C3, C4, and tryptase).

Table 9.1.2.1: Brown's Severity Criteria

Brown's Criteria for Hypersensitivity Reactions	Definition
Mild (1) skin and subcutaneous tissue	Generalized erythema, urticaria, periorbital edema, or angioedema
Moderate (2) features suggested respiratory, cardiovascular, or gastrointestinal involvement	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness (pre-syncope), diaphoresis, chest or throat tightness, or abdominal pain
Severe (3) hypoxia or neurologic compromise	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, loss of consciousness, or incontinence

Vial and syringe dose to prefilled syringe dose conversion table:

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
0 – 3.7	2.5	1	0	0
3.8 – 6.2	5	2	--	--
6.3 – 8.7	7.5	3	--	--
8.8 – 11.2	10	--	1	--
11.3 – 13.7	12.5	1	1	--
13.8 – 16.2	15	2	1	--
16.3 – 18.7	17.5	3	1	--
18.8 – 21.2	20	--	--	1
21.3 – 23.7	22.5	1	--	1
23.8 – 26.2	25	2	--	1
26.3 – 28.7	27.5	3	--	1
28.8 – 31.2	30	--	1	1
31.3 – 33.7	32.5	1	1	1
33.8 – 36.2	35	2	1	1
36.3 – 38.7	37.5	3	1	1
38.8 – 41.2	40	--	--	2
41.3 – 44.9	42.5	1	--	2
45 – 54.9	50	--	1	2
55 – 64.9	60	--	--	3
65 – 74.9	70	--	1	3
75 – 84.9	80	--	--	4

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
85 – 94.9	90	--	1	4
95 – 104.9	100	--	--	5
105 – 114.9	110	--	1	5
115 – 124.9	120	--	--	6
125 – 134.9	130	--	1	6
135 – 144.9	140	--	--	7
145 – 154.9	150	--	1	7

[a] For < 45 mg/day, round to nearest 2.5 mg; for ≥ 45 mg/day, round to nearest 10 mg.

BIOMARIN®

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria (PKU)
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Monitor:	[redacted], MD [redacted], Clinical Sciences BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	Up to 102 months or until study is terminated
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg or 375 mg/week
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011
Date of Amendment 4:	June 7, 2012
Date of Amendment 5:	February 28, 2014
Date of Amendment 6:	October 30, 2014
Date of Amendment 7:	December 16, 2015

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 3

Date: 16 December 2015

RATIONALE AND SUMMARY OF CHANGES

This section provides a numbered list of all significant changes and supporting rationale.

1. Collection of skin reaction data is being added to aid in assessment of skin reaction adverse events (AEs). The Skin Reaction electronic Case Report Form (eCRF) should be completed for skin reactions that last 14 days or longer. Photographs of the skin reactions may also be collected.
2. 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. Because of the long-term nature of this study, subjects may re-start study drug following birth and cessation of breastfeeding (if applicable) per the investigator and the sponsor. Information regarding contraception use has also been added.
3. The inclusion criterion regarding contraception use for subjects who are sexually active has been revised. Subjects must now use two acceptable methods of contraception while participating in the study to align with the contraception requirements in the Phase 3 studies.
4. The study duration has been extended from 98 months to 102 months.
5. The AE reporting period has been clarified; nonserious AEs and serious AEs (SAEs) should continue to be reported through 4 weeks after the last dose of study drug or the Study Completion Visit/Early Termination Visit, whichever occurs last.
6. Minor changes have been made to improve clarity and consistency.

In addition, the Schedule of Events and Section 12 have been updated to reflect the changes in this amendment. Refer to Section 24 for a summary of the amendment revisions.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data were collected for subjects who have already achieved Phe reduction to within the protocol-defined target range. This substudy has been completed as of 20 December 2013.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

STUDY DESIGN AND PLAN:

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first dose will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Additionally, subjects will be given two epinephrine injectors and will be instructed to carry one epinephrine injector with them at all times. A follow-up telephone call will be made to the subject to monitor

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Additional training will be provided to subjects and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Month 98 visit.
- The study is terminated.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

- The study drug receives marketing authorization.

Because the risks of taking rAvPAL-PEG during pregnancy and breastfeeding are unknown, subjects cannot take rAvPAL-PEG if they are trying to conceive, are pregnant, or are breastfeeding. Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study will be discontinued immediately from study drug. Male subjects who have impregnated a female partner may re-start study drug after conception but must return to the study-required contraception use, which must include one barrier method. Subjects who remain in the study after discontinuation of study drug due to pregnancy may re-introduce rAvPAL-PEG dosing after the birth has been reported (or termination of the pregnancy) and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting rAvPAL-PEG dosing must be per investigator and sponsor agreement based on an assessment of the known risks and potential benefits while taking BMN 165. Female subjects must return to the study-required contraception use, which must include one barrier method, immediately after the birth (or termination of the pregnancy).

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

PAL -003 Substudy

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed. This substudy has been completed as of 20 December 2013.

Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows.

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

Safety Assessment Criteria:

Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality. Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG.

Once an AE (other than anaphylaxis) improves to grade 1 or resolves, study drug dose may be increased, maintained, or reduced. If dosing has been interrupted due to an AE (other than anaphylaxis) and the investigator determines it is safe for the subject to resume dosing, the first dose after improvement of the AE should be performed in the clinic. The subject must be observed for 30 minutes following administration due to restarting dosing. The subject may be advised to premedicate with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated.

Stopping Criteria:

If an NCI-CTCAE grade $\geq$3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

NUMBER OF SUBJECTS PLANNED:

Up to 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

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- Must have completed participation in a previous rAvPAL-PEG study.
- Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use two acceptable methods of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
- Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
- A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's

NAME OF COMPANY	SUMMARY TABLE	FOR NATIONAL AUTHORITY USE ONLY:
BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

medical monitor. However, subjects who discontinued from study drug early due to a reaction are not eligible to enroll into this study.

- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
- Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or:

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or:

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- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Fixed Dosing

Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week).

DURATION OF TREATMENT:

Up to 102 months.

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

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Immunogenicity:

The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, anti-rAvPAL IgE, and anti-PEG-PAL IgE) will be assessed.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in a previous rAvPAL-PEG study may be enrolled into this study.
No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided.

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BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	Referring to Part of the Dossier: Volume: Page: Reference:	

Descriptive statistics for vital signs, physical examination results, ECG results, X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the Statistical Analysis Plan [SAP]) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

Details regarding exploratory analyses will be provided in the SAP.

Substudy Analysis:

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

3 TABLE OF CONTENTS

LIST OF TABLES

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice

GGT	gamma-glutamyltransferase
GI	gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	identification
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NOAEL	no observable adverse effect level
NSAID	non-steroidal anti-inflammatory medication
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell

SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Pharmacovigilance Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively,

and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given ≥ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.

7.2.1 Phase 1 Study, PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.
- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002, PAL-004, and 165-205

Based on results from the Phase 1, single-dose study, the Phase 2 studies have been designed to evaluate rAvPAL-PEG at various doses and dosing regimens to safely achieve and maintain blood Phe reductions in subjects with PKU. Currently, there are three ongoing Phase 2 studies (PAL-002, PAL-004, 165-205); an overview of each study is presented in Section 7.2.2.1 (PAL-002), Section 7.2.2.2 (PAL-004), and Section 7.2.2.3 (165-205).

7.2.2.1 Study PAL-002

Study PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria) was initiated in September 2009 and has completed enrollment. As of 10 September 2012, 40 subjects have been enrolled and 37 have completed the study. PAL-002 enrolled 11 previously exposed subjects from the single-dose study, PAL-001, and 29 subjects who were naïve to rAvPAL-PEG. Thirty-three of the 37 subjects who completed PAL-002 enrolled into this open-label extension study (PAL-003). Two subjects discontinued from the study due to lost to follow-up, relocation, or other reasons, and 1 subject discontinued from the study due to an AE (skin reaction).

The primary objective of this study was to evaluate the effect of multiple doses of rAvPAL-PEG (ranging from 0.001 mg/kg/week to 1.0 mg/kg/week) on blood Phe concentrations in subjects with PKU with up to 16 weeks of treatment. The secondary objectives of the study were to evaluate the safety and tolerability of SC injections of multiple doses of rAvPAL-PEG, to evaluate the immune response to rAvPAL-PEG, and to evaluate the PK profile of rAvPAL-PEG in subjects with PKU.

The PAL-002 study design consists of two parts. In Part 1, rAvPAL-PEG was administered as a once weekly fixed, low-dose induction regimen for 8 weeks. In Part 2, the rAvPAL-PEG dose was titrated upwards; subjects received adjustable dose increases for up to 8 weeks to achieve a target blood Phe concentration of 600 µmol/L. The doses and dosing schedules were revised with a series of protocol amendments to incorporate the information gained during conduct of this early, open-label, multiple-dose study.

A wide range of doses was planned for the PAL-002 study, beginning with doses as low as 0.001mg/kg/week. However, no substantial reductions in blood Phe level were observed in the majority of subjects who were administered rAvPAL-PEG in the initial four cohorts at doses of 0.001, 0.003, 0.01, and 0.03 mg/kg administered once per week. The absence of appreciable Phe reduction after 16 weeks of treatment in this range of doses led to a decision to amend the study protocol to include a cohort with a higher starting dose of 0.1 mg/kg/week, which had been previously shown to decrease blood Phe levels to approximately 600 µmol/L in the single-dose, Phase 1 study (PAL-001). After the first 2 weeks of dosing at 0.1 mg/kg/week, transient Phe reduction was observed in this subset of subjects. However, this dosing regimen was accompanied by mild to moderate hypersensitivity reactions primarily after the second weekly dose, suggesting that additional exploration of dosing regimens would be required.

In summary, preliminary results from PAL-002 demonstrated that doses below 0.1 g/kg administered once per week were not effective in reducing Phe levels. While transient reduction of Phe was apparent at doses of at least 0.1 mg/kg given once per week, this dose regimen was associated with a high incidence of systemic hypersensitivity reactions, typically following the second weekly dose. Further escalation above this dose of 0.1 mg/kg/week would be required to sustain the effect on Phe levels over time. Increased doses would require spreading dose administration over several days per week, as once-weekly administration of higher doses would not be practical due to the large volume of study drug. Therefore, additional dosing regimens would need to be explored in a subsequent study (PAL-004).

Once subjects completed Study PAL-002, they were eligible to enroll into this open-label extension study, PAL-003 to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.2 Study PAL-004

PAL-004 (An Open-Label Study to Evaluate the Safety, Tolerability, and Efficacy of Subcutaneous Dose Levels of rAvPAL-PEG Administered Daily in Subjects With Phenylketonuria) was initiated in April 2011 and has completed enrollment. As of 10 September 2012, 16 subjects have been enrolled, 15 subjects have completed the study, and 15 subjects have enrolled into this study (PAL-003). One subject withdrew consent from continued participation prior to study completion. All subjects enrolled in this study were naïve to rAvPAL-PEG exposure.

The objective of this study was to determine if daily administration (defined as 5days/week) of rAvPAL-PEG at dose levels of 0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day was safe and effective in reducing and maintaining blood Phe concentrations to 600 µmol/L in subjects with PKU. The starting dose was 0.4 mg/kg, administered 5 days/week. A total of 12 subjects were added in sequence and were started on doses of 0.4, 0.2, 0.1, 0.06 mg, or 0.001/kg/day based on incoming safety data. Overall, subjects in PAL-004 who initiated treatment at higher daily doses of 0.1 to 0.4 mg/kg/day achieved immediate and substantial reduction of blood Phe levels, but dosing had to be temporarily reduced or interrupted due to the onset of systemic hypersensitivity reactions at approximately Day 10 of dosing. Phe reductions did not persist in the setting of temporary dose reduction or interruption, but Phe levels generally improved if higher dose levels were reinstated.

To prevent the onset of hypersensitivity reactions that were temporally associated with the onset of anti-drug IgM responses, an additional dosing strategy was assessed in PAL-004;

4 subjects initiated dosing at a low dose of 0.001mg/kg/day or 0.06mg/kg/day for 5 consecutive days and then dosing was suspended for 2 weeks. Dosing was restarted on Day 21 at the initial dose followed by dose titration to target Phe levels. Subjects administered this dosing regimen had similar efficacy, but the incidence of hypersensitivity reactions appeared to be similar to that of subjects who did not have the planned dose interruption, suggesting little advantage with this alternate induction regimen.

Experience from PAL-004 indicated that initiating daily rAvPAL-PEG dosing with relatively high daily doses was not sustainable due to the onset of hypersensitivity reactions that occurred approximately 9 to 12 days after the start of administration, nor was the alternate dosing regimen (2 week drug holiday) well tolerated or effective in Phe reduction. In addition, the daily treatment regimen did not reduce the time to achieve target blood Phe levels seen in previous studies. This study indicated that to improve tolerability, rAvPAL-PEG should be initiated with an induction period at doses substantially lower than 0.4mg/kg, followed by upward dose titration toward target Phe levels.

Once subjects completed Study PAL-004, they were eligible to enroll into this open-label extension study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.3 Study 165-205

Study 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy, and Tolerability of Subcutaneously Administered rAvPAL-PEG in Subjects With PKU for 24 Weeks) was initiated in May 2012. Data from ongoing Studies PAL-002, PAL-004, and PAL-003 suggested that a 4-week treatment course with weekly dosing at a fixed low dose (induction), followed by a weekly 2-fold, upward titration of rAvPAL-PEG to approximately 10-fold higher than the initiation dose (maximum of 375 mg/week) is effective and well tolerated. The objective of this study was to further assess this dosing regimen. Two rAvPAL-PEG dosing regimens were to be explored in this study: weekly low-dose induction (2.5 mg/week for 4 to 8 weeks), followed by a period of upward dose titration towards a target Phe level (600 µmol/L) followed by maintenance dosing at that level through the 24 week study duration (Group 1). The other dosing regimen planned for this study (Group 2) involved administration of a single bolus dose of 8 mg, followed by a treatment holiday of at least 3 weeks duration followed by resumption and escalation of study drug administration following a pattern similar to that employed in Group 1. A total of 24 subjects were enrolled into 165-205. In addition to confirming an effective, well-tolerated dose regimen, this study incorporated non-weight-based dosing, starting with dose

administration once per week at a low dose (2.5mg) with gradual escalation and conversion of dose administration to 5x/week dosing for chronic maintenance therapy.

Once subjects have completed Study 165-205, they are eligible to enroll into this study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week, or into the Phase 3 study, 165-302.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an

exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

This study is an extension of previous rAvPAL-PEG Phase 2 studies. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from previous rAvPAL-PEG studies will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to Polyethylene Glycol (PEG)

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.

7.4.2 Immunologic Response

The active drug substance in rAvPAL-PEG is a bacterial protein and elicits immune recognition and subsequent antibody responses. Antibody epitopes on the protein are expected to be rendered at least partially inaccessible by the extensive PEGylation on the drug (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in reducing anti-rAvPAL antibody titers in the mouse model but does not eliminate antibody formation. Although PEGylation of rAvPAL likely reduces the antigenicity of the molecule, 100% of individuals exposed to rAvPAL-PEG develop antibodies against rAvPAL.

Drug-specific immune responses can range from clinical irrelevance to causing serious hypersensitivity AEs (such as anaphylaxis) and may reduce efficacy due to antibody-mediated drug clearance or antibody neutralization of enzymatic activity. Historically, PEG was thought to be nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.); however, repeated studies have found that subcutaneous (SC) and intravenous exposure to PEG can induce anti-PEG antibodies (Harris, 2003, Nat.Rev.Drug Discov.), (Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). However, PEG antibody responses were associated with hypersensitivity reactions to PEGylated liposomes (Judge, 2006, Mol.Ther.). Anti-PEG antibodies have also been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.).

Subjects participating in the Phase 2 clinical studies have been monitored closely for anti-drug immunogenicity (anti-rAvPAL immunoglobulin G [IgG], anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, neutralizing antibodies that inhibit PAL enzymatic activity, anti-rAvPAL IgE, and anti-rAvPAL-PEG IgE), complements, C-reactive protein (CRP), ESR, and complete chemistry. All subjects treated with rAvPAL-PEG in the Phase 2 clinical trials developed antibodies against the PAL protein. Anti-rAvPAL IgM antibodies were first detected within 2-3 weeks of treatment initiation and anti-rAvPAL IgG antibodies were detected within 2-3 months. The anti-rAvPAL IgG response generally peaked by 3-5 months and was sustained in all subjects. Neutralizing antibodies (NAb) capable of inhibiting enzymatic activity were detected in a minority of subjects 2-3 months after treatment initiation. The timing of the NAb response suggests these antibodies may be a component of

the anti-rAvPAL IgG response. It is expected that the centralized location of the PAL active site, within the tetrameric structure, makes it difficult for antibodies to bind and inhibit enzymatic activity. In addition, anti-PAL antibody binding is likely sterically hindered by the extensive PEGylation. The majority of treated subjects also developed a transient anti-PEG antibody response. The anti-PEG response was comprised of IgM and IgG isotypes. In general, anti-PEG antibodies were no longer detected after 5-6 months of treatment. This type of transient antibody response is typical of T cell-independent type 2 (TI-2) responses.

The majority of immune-mediated AEs experienced in subjects treated with rAvPAL-PEG in the Phase 2 studies are believed to be the result of antibody-mediated hypersensitivity. The primary immune mediator is thought to be IgM immune complexes, as evidenced by the timing of onset prior to IgG development and the reduced occurrence over time. IgE positive test results have not been associated with hypersensitivity in any of the subjects thus far, and the majority of subjects have been safely rechallenged with rAvPAL-PEG following reactions. IgM immune complexes are known to be efficient activators of the classical complement pathway and can lead to the accumulation of complement component anaphylatoxins (C3a, C4a, C5a) that can induce hypersensitivity symptoms. Although IgG immune complexes can also activate the classical complement pathway, they do so with less efficiency than IgM. This is due, in part, to the requirement for multiple Fc regions in close proximity to activate the complement cascade, meaning that multiple monomeric IgG Abs are required to bind, whereas only one pentameric IgM Ab is needed to bind to activate complement. Also, in the case of rAvPAL-PEG, binding of anti-PAL IgG antibodies is likely compromised due to epitope masking by the PEGylation, which may reduce IgG-mediated complement activation *in vivo*. This complex dynamic between combined isotype-specific Ab responses and epitope accessibility is thought to be a major factor dictating the need for an induction and titration dose regimen in the treatment of rAvPAL-PEG to be implemented in this study.

7.4.3 Management of Hypersensitivity Reactions

Hypersensitivity reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30 minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of hypersensitivity reactions. Because of the potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection. Subject should be pre-medicated with H1 antagonist, H2

antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated. Subjects who qualify for self-administration of study drug are trained to recognize a serious hypersensitivity AEs and how to respond. Additionally, subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (Section 9.4). Refer to Section 9.1.1.3 for instructions regarding resumption of study drug following resolution of symptoms consistent with a clinical diagnosis of anaphylaxis per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria (Sampson, 2006, J.Allergy Clin.Immunol.).

In the event of a hypersensitivity reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of hypersensitivity reaction symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oral or IV glucocorticoids.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. An allergy and/or immunology consultation should be sought if necessary. Detailed instructions for the management of hypersensitivity reactions are provided in the Study Reference Manual.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objective is as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity was observed in a previous rAvPAL-PEG studies. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first week of dosing will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (refer to Section 9.4). A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to clinic monthly for review with the clinical study staff.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self-administration. Additional information is provided in the Subject Self-Administration Training Materials. Additional training will be provided and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 9.1.1 and Table 9.1.2. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Month 98 visit.
- The study is terminated.
- The study drug receives marketing authorization.

Because the risks of taking rAvPAL-PEG during pregnancy and breastfeeding are unknown, subjects cannot take rAvPAL-PEG if they are trying to conceive, are pregnant, or are breastfeeding (refer to Section 9.3.2, Exclusion Criteria). Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study will be discontinued immediately from study drug. Male subjects who have impregnated a female partner may re-start study drug after conception but must return to the study-required contraception use (refer to Section 9.3.1, Inclusion Criteria), which must include one barrier method. Subjects who remain in the study after discontinuation of study drug due to pregnancy may re-introduce rAvPAL-PEG dosing after the birth has been reported (or termination of the pregnancy) and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting rAvPAL-PEG dosing must be per investigator and sponsor agreement based on an assessment of the known risks and potential benefits while taking BMN 165. Female subjects must return to the

study-required contraception use (refer to Section 9.3.1), which must include one barrier method, immediately after the birth (or termination of the pregnancy).

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2).

The Schedule of Events and PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2.

Table 9.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]						Self-Administration	Study Completion/Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly [b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)					
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures				4 weeks after final dose	
Informed consent	X									
Medical history, including allergy history, and demographics	X									
Physical examination[e]	X	X			X				X	
Vital signs[e]	X	X		X					X	X
Weight	X			X						
12-lead ECG	X								X	
Clinical laboratory tests[f]	X			X					X	X[g]
Complements C_3 and C_4[h]	X				X					X[g]
Sedimentation rate		X			X				X	
Chest x-ray	X			X (Week 48 visit only)					X	
Urine pregnancy test[i]	X	X		X					X	
Adverse events[j,k]	X	X	X	X				X	X	X
Weekly phone call to self admin participants only (to assess AEs, Inj site reactions, concomitant medications)			X					X		
Concomitant medications	X	X	X	X				X	X	X
Diet query	X	X		X					X	
3-Day diet diary[l]				X					X	
Serum antibodies[m]		X			X				X	X

Assessments and Events	Screening[a]	Treatment Period[b,c]						Self-Administration	Study Completion/Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Monthly[b] (eg, Wk 4, 8, etc.)	Quarterly (eg, Wk 12, 24, etc.)					
		D 1	Starting at Week 2	Every 4th week;	Every 12th week. Perform in addition to monthly procedures				4 weeks after final dose	
Plasma Phe and plasma tyrosine[n]	X	X		X[m]					X	
Urine albumin/creatinine ratio										X
Urine N-methyl histamine										X
Plasma PK sample[o]		X			X				X	
Administer study drug[p]		X		X				X		
Training (prefilled syringe)[q]				X Transition to prefilled syringe	X Every 24 weeks only					
Skin biopsy (optional; affected and not affected area)										X
Serum tryptase level[r]										X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; eCRF, electronic Case Report Form; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] A separate Screening visit for this study is required only if the time between completion of the previous rAvPAL-PEG study and enrollment into PAL-003 is greater than 28 days.

[b] Monthly visits must be performed in the clinic.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Refer to Section 9.1.1 and Section 12.3.6.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis. Urine microscopy will be performed if any urinalysis results are positive for hematuria. Refer to Table 9.7.6.1.

[g] Subjects who have a National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade ≥ 3 hypersensitivity AE should be assessed for CRP, CH50, C1, C3, and C4 within 24 hours of the reaction.

[h] Complement C3 and C4 will be collected at the Screening Visit and quarterly. Additional complement testing will be performed and as needed to resolve previous abnormal test results.

[i] If positive or equivocal, perform serum pregnancy test. Also refer to Section 9.1.

[j] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm). If there is a skin reaction that lasts ≥ 14 days, the Skin Reaction eCRF should be completed. A photograph of the skin reaction may be taken by the subject or the site to help assess the event and may be collected by the sponsor.

[k] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or completion of study participation, whichever occurs last. The reporting period for nonserious AEs is from the first administration of study drug through 4 weeks after last dose or the Study Completion Visit/Early Termination Visit, whichever occurs last. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.

[m] For unscheduled hypersensitivity reaction visit, draw serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only; sampling must be performed > 8 hours after event onset and before the next dose of study drug.

[n] Samples should be drawn at least 2.5 hours after a meal. At the Investigator's discretion, blood Phe may be collected more often should more frequent monitoring of blood Phe levels be clinically warranted.

[o] Sampling should be performed predose.

[p] Dosing is up to 5.0 mg/kg/week or 375 mg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may be re-evaluated.

[q] Initial training on the use of prefilled syringe occurs at the next monthly visit and each subsequent day after transition until (in clinic or by HHRN) it has been determined the subject will switch to prefilled syringe. Once competency is documented, subjects can self-administer study drug using the prefilled syringe. Regular refresher prefilled syringe training occurs in the study clinic every 24 weeks thereafter.

[r] Mandatory for subjects who have a hypersensitivity AE. Perform within 24 hours of event onset.

Table 9.1.2: PK Substudy Dosing Regimens

Treatment Frequency	Plasma PK Sampling	Example
Subjects who are administered study drug once per week	Pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours when possible), and pre-dose of the next weekly dose	If dosed on Monday: Obtain PK sample pre-dose when possible – pre-dose Monday, Tuesday, Wednesday, Thursday, Friday, and pre-dose the following Monday. No further PK substudy draws.
Subjects who are administered study drug two or three times per week	Pre-dose and every 24 hours during the longest period between doses, when possible, and pre-dose of the next dose.	If dosed on Monday, Thursday, and Friday: Obtain PK sample every 24 hours during the longest period between doses: Pre-dose Monday, Tuesday, Wednesday, and pre-dose Thursday. No further PK substudy draws.
Subjects who are administered study drug four to seven times per week.	Pre-dose and every 12 hours during the longest period between doses, when possible, then pre-dose of the next dose.	If dosed Monday through Friday: Obtain PK sample every 12 hours during the longest period between doses (Friday to Monday): pre-dose Friday, 12 hours post-dose Friday, Saturday (24 and 36 hours post dose), Sunday (48 and 60 hours post-dose), and pre-dose the following Monday. No further PK substudy draws.

9.1.1 Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. NSAIDs should be given with food and may be omitted if not tolerated.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality (Section 9.1.1.3). Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

9.1.1.1 Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

9.1.1.2 Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG. Dosing instructions are presented in Table 9.1.1.2.1 and are regardless of previous occurrence:

Table 9.1.1.2.1: Dosing Instructions in Response to Hypersensitivity Adverse Events

NCI-CTCAE Grade[a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria [d]	HRV Assessment[e]
		Maintain[b]	Reduce[c]	Interrupt[c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No	X	(X) Optional	(X) Optional		Investigator discretion
3	Yes	X	(X) Optional	(X) Optional		Yes
3 and is suspected to meet Brown's criteria for severe [d]	Yes				X Consult with sponsor medical monitor	Yes
4 [d]	Yes or No				X Consult with sponsor medical monitor	Yes

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, version 4.03; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the subject to maintain the rAvPAL-PEG dose at the time of AE onset until improvement to grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The rAvPAL-PEG dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE grade ≥3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor, the subject may be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE grade ≥3 hypersensitivity AE is related to administration with rAvPAL-PEG, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE [sampling must be performed >8 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, urinary N-methyl histamine, CRP, C3, C4, and tryptase).

9.1.1.3 Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and NSAIDs approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. NSAIDs should be given with food and may be omitted if not tolerated.

9.1.2 Study Stopping Criteria

If an NCI-CTCAE grade ≥ 3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

Brown's severity criteria are presented in Table 9.1.2.1.

Table 9.1.2.1: Brown's Severity Criteria

Brown's Criteria for Hypersensitivity Reactions	Definition
Mild (1) skin and subcutaneous tissue	Generalized erythema, urticaria, periorbital edema, or angioedema
Moderate (2) features suggested respiratory, cardiovascular, or gastrointestinal involvement	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness (pre-syncope), diaphoresis, chest or throat tightness, or abdominal pain
Severe (3) hypoxia or neurologic compromise	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, loss of consciousness, or incontinence

9.1.3 Dose Modifications

9.1.3.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

9.1.3.2 Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.

4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use two acceptable methods of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's medical monitor. However, subjects who discontinued from study drug early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study drug should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study drug include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy and refer to Section 9.1 for details on re-introduction of study drug following pregnancy and breastfeeding).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in their previous rAvPAL-PEG study. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose that was administered upon completion of that study provided that there was no interruption in dosing. rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be adjusted per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects may be eligible to self-administer study drug if he or she meets the following criteria:

- The subject has no cognitive impairments that may increase the safety risk of self-administration per the assessment of the investigator.
- The subject has been approved for self-administration of study drug by the sponsor's medical monitor.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with two epinephrine injectors and has been trained on when and how to administer it. The subject will be instructed to carry one epinephrine injector with them at all times.

Qualified study site personnel will train each eligible subject on all procedures for self-administration of study drug (vial and syringe and prefilled syringe drug products) under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self-administer a dose at home. The subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 9.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials. Prefilled syringes are dispensed to subjects only after the subject (or caregiver) has completed training and competency is demonstrated in the clinic. Additional regular refresher training occurs in the clinic, every 24 weeks, and more often as needed.

Subjects who are eligible for self-administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.
- How to safely use and dispose of syringes, including prefilled syringes, used for injections of study drug.
- How to use a new syringe and vial or new prefilled syringe every time drug is administered.

- How to care for their injection site after an injection of study drug.
- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine injectors.
- Who to contact at the study site in case of an emergency.

The PI or the sponsor's medical monitor may request that self-administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplying the study drug to subjects who qualify for self-administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self-administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ± 0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Table 9.4.2.1.1: Prefilled Syringe Volume, Concentration, and Dose

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

Subjects should convert their vial and syringe dose to prefilled syringe dose using the following table:

Table 9.4.2.1.2: Conversion from Vial and Syringe Dose to Prefilled Syringe Dose

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
0 – 3.7	2.5	1	0	0
3.8 – 6.2	5	2	--	--
6.3 – 8.7	7.5	3	--	--
8.8 – 11.2	10	--	1	--
11.3 – 13.7	12.5	1	1	--
13.8 – 16.2	15	2	1	--
16.3 – 18.7	17.5	3	1	--
18.8 – 21.2	20	--	--	1
21.3 – 23.7	22.5	1	--	1
23.8 – 26.2	25	2	--	1
26.3 – 28.7	27.5	3	--	1
28.8 – 31.2	30	--	1	1
31.3 – 33.7	32.5	1	1	1
33.8 – 36.2	35	2	1	1
36.3 – 38.7	37.5	3	1	1
38.8 – 41.2	40	--	--	2
41.3 – 44.9	42.5	1	--	2
45 – 54.9	50	--	1	2
55 – 64.9	60	--	--	3
65 – 74.9	70	--	1	3
75 – 84.9	80	--	--	4
85 – 94.9	90	--	1	4
95 – 104.9	100	--	--	5
105 – 114.9	110	--	1	5
115 – 124.9	120	--	--	6
125 – 134.9	130	--	1	6
135 – 144.9	140	--	--	7
145 – 154.9	150	--	1	7

[a] For <45 mg/day, round to nearest 2.5 mg; for ≥45 mg/day, round to nearest 10 mg.

The excipients in this drug product wre tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60 600 µmol/L), as well as any adverse events (any hypersensitivity AE) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

For vial and syringe, drug product packaging will be identified with the lot number and expiration date and will be provided in a box labeled with the study number.

For prefilled syringe, drug product packaging will be identified with the size (2.5 mg, 10 mg, or 20 mg), lot number, and kit identification (ID). The expiration date will be available in a separate Certificate of Compliance issued for each lot.

9.4.3 Storage

IP must be stored at 5 ± 3°C (41 ± 5°F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose).

For subjects receiving weight-based dosing, dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to

accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1.

Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 15 mg/ml concentration (ml) (vial)[a]
0.03	2.5	0.17
0.06	5	0.33
0.12	10	0.67
0.25	20	1.33
0.5	40	2.67
1.0	75	5
2.0	150	10
3.0	225	15
4.0	300	20
5.0	375	25

[a] Applies to both 1.0 and 1.3 ml withdrawal.

It is preferable that the injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff, other qualified and trained study personnel, or qualified subjects.

Information for dosing of subjects who enroll into this study after completing previous rAvPAL studies is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in their previous rAvPAL-PEG study.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self-administer study drug at home if approved by the sponsor's medical monitor and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the sponsor's medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or

requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product will be encouraged to be premedicate as outlined in Section 9.1.1. If the hypersensitivity AE worsens with a repeat injection (as determined by the Investigator in consultation with the sponsor's medical monitor) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.3.2).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for dosing of subjects who enroll into this study after completing previous rAvPAL-PEG studies is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator. After a dosing interruption of ≥ four consecutive doses, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first week of dosing of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30 minutes following administration due to restarting dosing. Premedication with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2-3 hours prior to study drug should be administered following any dose interruption of ≥ four consecutive doses. NSAIDs should be given with food and may be omitted if not tolerated. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects

should continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1) during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study drug per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self-administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next monthly study visit. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a] or CRO	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Pregnancy test (urine) and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1
Urinalysis	Central laboratory	9.7.6

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL IgE and anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn monthly, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1).

Blood Phe may be collected more often than monthly at the discretion of the Investigator should more frequent monitoring of blood Phe levels be clinically required.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed. For subjects participating in the Substudy, PK sampling will be performed as follows:

- For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.
- For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose.
- For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

See Table 9.1.2 for additional details.

BioMarin will perform the analysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1).

BioMarin or CRO will perform all anti-body testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Phenylalanine
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Sedimentation rate[a]
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Spot urine albumin/creatinine ratio	**Additional Unscheduled Hypersensitivity Reaction Visit Tests[b]**
Total protein		Nitrite	CH50
Calcium		Urobilinogen	C_1, C_3, C_4
Sodium		Hemoglobin	Serum tryptase level[b]
Potassium		Bilirubin	CRP
Glucose			Urine N-methyl histamine
			Urine albumin/creatinine ratio
Uric acid			**Complement Testing[c]**
CO_2			C_3
Chloride			C_4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory.

[b] Perform within 24 hours of event onset.

[c] Complement C_3 and C_4 to be drawn at the Screening Visit and then quarterly or as needed to resolve abnormal test results.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study AE reporting period is as follows: After informed consent but prior to initiation of study drug, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, all non-serious AEs and SAEs reporting period begins and continues until 4 weeks following the last administration of study drug or the Study Completion Visit/Early Termination Visit, whichever occurs last (refer to Section 12). The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

For this study, a medical device is defined as the prefilled syringe and all of the component parts. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe. Events assessed by the investigator as related to the device, including malfunction, injury, or medication error, will be captured into an electronic data capture system. All serious events related to the device will be reported to BioMarin Pharmacovigilance (BPV) within 24 hours using the device-related event report form and entered onto the appropriate eCRF page(s) as required.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study drug or study participation is not the cause of the AE. Outcome of AEs (and resolution dates) should be documented on the appropriate CRF page(s) and in the subject's medical record unless the subject is lost to follow-up or it has been determined that the study drug or study participation is not the cause of the AE.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)[b]
4	Life threatening or debilitating: consequences; urgent intervention indicated
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	Description
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
    - Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.

- Important medical events or reactions that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes above should also usually be considered serious.

For this protocol, anaphylaxis per NIAID/FAAN criteria for the clinical diagnosis of anaphylaxis is designated as an AE of special interest (serious or nonserious and irrespective of severity) to facilitate rapid reporting and sponsor review. All occurrences of anaphylaxis per NIAID/FAAN criteria will be reported to the sponsor within 24 hours of the site becoming aware of the event using the SAE form. Severity and serious criteria (if applicable) should be reported on the SAE form.

The reporting period for SAEs related to protocol imposed interventions begins after informed consent is obtained, the reporting period for all SAEs begins after first dose of study drug and continues until 4 weeks following the last administration of study drug or End of Treatment Visit. The reporting period for Serious Device Related Events begins after first dose of study drug and ends with the last administered dose.

All SAEs (including serious device-related events), whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form or device Report Form to BioMarin Pharmacovigilance (BPV). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study drug or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the subject's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

At the last scheduled visit, the Investigator should instruct each subject to report any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study drug.

The Investigator should notify the study Sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior

study drug. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study.

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the End of Study CRF if the subject withdraws from the study due to pregnancy. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution to BPV in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman's consent for release of protected health information.

10.4 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (i.e., modified dose amount or frequency not defined in protocol).
- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.5 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the Sponsor's Medical Office sponsor's medical monitor r any AEs for which the issue of seriousness is unclear or questioned. Contact information for the sponsor's medical monitor is as follows:

Name: [redacted], MD
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to clinic on a monthly basis for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis)
- Complement C_3 and C_4
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications

assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. If there is a skin reaction that lasts ≥14 days, the Skin Reaction eCRF should be completed. A photograph of the skin reaction may be taken by the subject or the site to help assess the event and may be collected by the sponsor.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator)

12.3.2 Weekly Telephone Visit

Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled monthly visits per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:

- Injection-site self inspection (previous and current injection site)
- Assessment of AEs

- Concomitant medications
- Confirmation of dose level, frequency, and study drug administration

12.3.3 Substudy Visits

For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2.

12.3.4 Monthly Visits (Week 4, 8, 12, etc)

Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 4, monthly visits must be performed in the clinic. The following study activities will be performed:

- Vital signs
- Weight
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray.
- Chest x-ray (Week 48 visit only)
- Assessment of AEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.)
- Prefilled syringe self-administration training (to be performed prior to subjects transitioning from vial and syringe drug product to prefilled syringe drug product).
- Administer study drug
- If additional training is needed on days subsequent to the monthly visit, additional clinic visit(s) or HHRN may be used.

12.3.5 Quarterly Visits (Week 12, 24, 36, etc)

Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Beginning with Week 12, quarterly visits consist of all monthly (Section 12.3.4) activities listed above as well as the additional study activities listed below:

- Physical examination
- Complement C_3 and C_4
- Sedimentation rate
- PK sample
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Prefilled syringe refresher training (every 24 weeks only)

12.3.6 Unscheduled Hypersensitivity Reaction Visit

Subjects who have an NCI-CTCAE grade ≥ 3 hypersensitivity reaction after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 24 hours) the reaction.

- Vital signs
- Clinical laboratory tests
- Urine/albumin creatinine ratio
- CRP, CH50, C1, C3, and C4
- Urine N-methyl histamine
- Serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only (performed > 8 hours after event onset)
- Serum tryptase level
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of the reaction)
- Assessment of AEs
- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects may resume study drug administration per the discretion of the Principal Investigator and the sponsor's medical monitor.

12.4 Study Completion/Early Termination Visit

Subjects should perform the Study Completion Visit 4 weeks after the last dose of study drug

or after completion of study participation, whichever occurs last.

For subjects who terminate from the study early (refer to Section 9.3.3), the Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug or after withdrawal from study participation, whichever occurs last. Subjects who terminate from study drug early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF).

The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics of vital signs, physical examination results, ECG results, and X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should

data become available from previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

14.3.1 Pharmacokinetic Substudy Analysis

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored. This substudy has been completed as of 20 December 2013.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the SAP) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis.

Details regarding exploratory analyses will be provided in the Statistical Analysis Plan.

14.5 Determination of Sample Size

Subjects who participated in previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Assess the effect and relevance of new external evidence.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.2.
- Make recommendations to the BioMarin concerning continuation or termination of the study or other study modifications based on observations.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment, unless the executed Clinical Trial Agreement between BioMarin and the institution of the investigator specifies otherwise. If the subject has followed the Investigator's instructions, BioMarin may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. Upon completion of the clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003, Amendment 7

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

[redacted] 16 DEC 2015

Medical Monitor Signature Date

Printed name: [redacted]

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following is a summary of significant protocol revisions; added text is underlined and deleted text is ~~struck~~. The study design schematic (Figure 9.1.1) and Schedule of Events (Table 9.1.1) were revised accordingly. Additional administrative changes have been made for consistency and clarity throughout this amendment and are reflected in the protocol body. Revisions made to the Synopsis (Section 2) are reflected in the corresponding protocol section as appropriate. Revisions to cross references to protocol tables, figures, sections, and references reside within the protocol body.

Section	Revision	Rationale for Change
Section 2, Section 9.1, Study Design	Because the risks of taking rAvPAL-PEG during pregnancy and breastfeeding are unknown, subjects cannot take rAvPAL-PEG if they are trying to conceive, are pregnant, or are breastfeeding. Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study will be discontinued immediately from study drug. Male subjects who have impregnated a female partner may re-start study drug after conception but must return to the study-required contraception use, which must include one barrier method. Subjects who remain in the study after discontinuation of study drug due to pregnancy may re-introduce rAvPAL-PEG dosing after the birth has been reported (or termination of the pregnancy) and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting rAvPAL-PEG dosing must be per investigator and sponsor agreement based on an assessment of the known risks and potential benefits while taking BMN 165. Female subjects must return to the study-required contraception use, which must include one barrier method, immediately after the birth (or termination of the pregnancy).	2
Section 2, Section 9.3.1, Inclusion Criteria	Individuals eligible to participate in this study must meet all of the following criteria: • Sexually active subjects must be willing to use ~~an~~two acceptable methods of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.	3
Section 2, Duration of Treatment	Up to 102~~98~~ months.	4
Section 9.1, Study Design	Because the risks of taking rAvPAL-PEG during pregnancy and breastfeeding are unknown, subjects cannot take rAvPAL-PEG if they are trying to conceive, are pregnant, or are breastfeeding (refer to Section 9.3.2, Exclusion Criteria). Male subjects who are planning to impregnate a female	2

Section	Revision	Rationale for Change
	partner and female subjects who are trying to become pregnant during the study will be discontinued immediately from study drug. Male subjects who have impregnated a female partner may re-start study drug after conception but must return to the study-required contraception use (refer to Section 9.3.1, Inclusion Criteria), which must include one barrier method. Subjects who remain in the study after discontinuation of study drug due to pregnancy may re-introduce rAvPAL-PEG dosing after the birth has been reported (or termination of the pregnancy) and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting rAvPAL-PEG dosing must be per investigator and sponsor agreement based on an assessment of the known risks and potential benefits while taking BMN 165. Female subjects must return to the study-required contraception use (refer to Section 9.3.1), which must include one barrier method, immediately after the birth (or termination of the pregnancy).	
Table 9.1.1, Schedule of Events	The table has been updated to reflect changes made with this amendment.	1, 5, 6
Section 9.3.3, Removal of Subjects from Treatment or Assessment	Reasons for which the Investigator or BioMarin may withdraw a subject from the study ~~treatment~~ drug include, but are not limited to, the following: • Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy and refer to Section 9.1 for details on re-introduction of study drug following pregnancy and breastfeeding).	2
Section 10.1, Adverse Events	…. After informed consent is obtained and the first administration of study drug, all non-serious AEs and SAEs reporting period begins and continues until 4 weeks following the last administration of study drug or the Study Completion Visit/Early Termination Visit, whichever ~~is longer~~occurs last (refer to Section 12). The criteria for determining, and the reporting of SAEs is provided in Section 10.2.	5
Section 10.3, Pregnancy	Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and	2

Section	Revision	Rationale for Change
	continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the End of Study CRF if the subject withdraws from the study due to pregnancy….	
Section 12.3, Treatment Period	During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. ~~All study activities will occur on the first day of each week unless otherwise noted.~~ On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. If there is a skin reaction that lasts ≥ 14 days, the Skin Reaction eCRF should be completed. A photograph of the skin reaction may be taken by the subject or the site to help assess the event and may be collected by the sponsor.	1
Section 12.4, Study Completion/Early Termination Visit	Subjects should perform the Study Completion Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early (refer to Section 9.3.3), t~~T~~he Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug or after withdrawal from study participation, whichever occurs last. …	5
Section 20, Use of Information and Publication	BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good	6

Section	Revision	Rationale for Change
	publication practices (GPP). ~~BioMarin recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the institution of the investigator.~~	

CLINICAL STUDY PROTOCOL

Study Title:	Long-term Extension of a Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU
Protocol Number:	PAL-003
Investigational Product:	rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase)
IND/EUDRACT Number:	IND 076269
Indication:	Phenylketonuria (PKU)
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 2
Sponsor's Responsible Medical Monitor:	[redacted] MS
Duration:	Approximately 119 months
Dose:	0.001 to a maximum weekly dose of 5.0 mg/kg or 375 mg/week
Date of Original Protocol:	October 08, 2008
Date of Amendment 1:	February 09, 2009
Date of Amendment 2:	October 30, 2009
Date of Amendment 3:	May 04, 2011
Date of Amendment 4:	June 7, 2012
Date of Amendment 5:	February 28, 2014
Date of Amendment 6:	October 30, 2014
Date of Amendment 7:	December 16, 2015
Date of Amendment 8:	**March 31, 2017**

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 8

Date: 31 March 2017

RATIONALE AND SUMMARY OF CHANGES

This section provides a numbered list of all significant changes and supporting rationale:

1. To monitor subject safety, a repeat urinalysis should be performed for subjects who have an abnormal or elevated proteinuria test result. Additionally, first or second morning void is being recommended for all urinalyses collected during the study. In addition, information regarding renal toxicity due to exposure to PEG has been updated in the background section (Section 7.4.1).
2. Additional instruction has been added for subjects who are pregnant or are planning to become pregnant (or impregnate a female partner) during the study for clarity and to monitor subject safety.
    - Subjects who are planning to conceive must stop study drug at least 4 weeks prior to trying to conceive and must use two forms of acceptable contraception prior to trying to conceive.
    - Subjects who are planning to become pregnant (or impregnate a female partner) during the study may modify their diet in consultation with the investigator and/or study dietician.
    - Subjects who are pregnant or are trying to conceive and have temporarily discontinued study drug are not required to perform the scheduled pharmacokinetic (PK) assessments.
    - Subjects who have been confirmed to be pregnant with a serum pregnancy test are not required to perform the urine pregnancy tests while pregnant.
    - Upon re-starting study drug following a pregnancy or breastfeeding, female subjects must have a confirmed negative urine pregnancy test result.
3. Additional instruction regarding adverse event (AE) and serious AE (SAE) reporting has been added to provide clarity and to improve the quality of safety reporting. The following have been added:
    - Guidance on factors suggestive of a causal relationship to study drug to facilitate investigator assessments of AEs
    - Guidance on how to report AEs occurring secondary to other events
    - Guidance on reporting of persistent versus recurrent AEs

- Guidance on when abnormal laboratory test results should be reported as AEs
- Guidance on how pre-existing conditions should be recorded
- Guidance on where and how physician examination findings should be reported as AEs
- Guidance on scenarios that do not require reporting hospitalization as an SAE
- Guidance on how to report deaths
- Guidance on how to report abortions
- Guidance on reporting post-study SAEs
- Guidance regarding sponsor request for additional case details for SAEs and AEs of special interest, including hospital discharge, consultant reports, and autopsy reports

4. To monitor for subject safety, subjects who have an elevated urine/albumin creatinine ratio (defined as confirmed result of ≥ 100 mg/g for subjects with results within normal range at baseline or a confirmed increase of 100-200 mg/g from baseline for subjects with elevated results at baseline) should be referred to a nephrologist for consultation.
5. To monitor for subject safety, subjects who experience an injection-site skin reaction that lasts ≥14 days should be referred for a dermatology consultation and may be asked to perform a skin biopsy.
6. Instruction regarding the premedications to administer prior to injection of study drug has been revised to provide alternative medications to subjects who may not tolerate non-steroidal anti-inflammatory drugs (NSAIDS); subjects may alternatively be premedicated with antipyretics (eg, acetaminophen).
7. Information regarding exploratory sample analysis (use of leftover blood and urine samples) during the study and after the subject completes the study has been added.
8. The Schedule of Events has been modified; scheduled visits are to occur every 8 weeks (rather than every 4 weeks and quarterly) to align with the visit schedule in the ongoing extension (Part 4) of the Phase 3 study, 165-302.
9. The study duration has been extended from 102 months to approximately 119 months.
10. The responsible Medical Monitor has been changed.
11. Minor changes were made to improve clarity and consistency and update protocol language to reflect more recent information and/or instruction regarding study conduct.

In addition, the Schedule of Events (Section 9.1) and Section 12 have been updated to reflect the changes in this amendment. Refer to Section 24 for a summary of the amendment revisions.

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase) NAME OF ACTIVE INGREDIENT: phenylalanine ammonia lyase	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**

TITLE OF STUDY:
Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

PROTOCOL NUMBER: PAL-003

STUDY SITES: Approximately 20 centers in the United States

PHASE OF DEVELOPMENT: Phase 2

STUDY RATIONALE:

The need exists for a treatment that will help individuals with phenylketonuria (PKU) safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits. This study is an extension of the rAvPAL-PEG Phase 2 studies PAL-002, PAL-004, and 165-205. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data were collected for subjects who have already achieved Phe reduction to within the protocol-defined target range. This substudy has been completed as of 20 December 2013.

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OBJECTIVES:

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objectives are as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

STUDY DESIGN AND PLAN:

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity is observed in a previous rAvPAL-PEG study. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first dose will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Additionally, subjects will be given two epinephrine injectors and will be instructed to carry one epinephrine injector with them at all times. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

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Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be reviewed with the clinical study staff on a monthly basis.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Additional training will be provided to subjects and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects' previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Week 476 visit.
- The study is terminated.
- The study drug receives marketing authorization.

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Because the risks of taking BMN 165 during pregnancy and breastfeeding are unknown, subjects cannot take BMN 165 if they are trying to conceive, are pregnant, or are breastfeeding (refer to Section 9.3.2 for Exclusion Criteria). Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study must be temporarily discontinued from study drug for 4 weeks prior to trying to conceive. During that time, subjects must use two acceptable methods of contraception. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician. Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests. Subjects who are pregnant or are trying to conceive and have temporarily discontinued study drug should not perform the scheduled PK assessments. Male subjects who have impregnated a female partner may re-start study drug after conception following the investigator's consultation with and approval by the medical monitor. Male subjects must return to the study-required contraception use, which must include one barrier method, prior to restarting study drug. Female subjects who remain in the study after temporary discontinuation of study drug due to pregnancy may restart BMN 165 dosing after a confirmed negative urine pregnancy test result, the birth has been reported (or termination of the pregnancy), and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting BMN 165 dosing requires the investigator's prior consultation with and approval by the medical monitor. Female subjects must return to the study-required contraception use, which must include one barrier method, immediately after the birth (or termination of the pregnancy).

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

PAL -003 Substudy

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed. This substudy has been completed as of 20 December 2013.

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Dose Modifications:

Dose Increase Methodology:

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4,0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events (any hypersensitivity reaction) for an individual subject. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

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A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

Safety Assessment Criteria:

Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. If non-steroidal anti-inflammatory medication (NSAIDs) is administered as a premedication, it should be given with food.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality. Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG.

Once an AE (other than anaphylaxis) improves to grade 1 or resolves, study drug dose may be increased, maintained, or reduced. If dosing has been interrupted due to an AE (other than anaphylaxis) and the investigator determines it is safe for the subject to resume dosing, the first dose after improvement of the AE should be performed in the clinic. The subject must be observed for 30 minutes following administration due to restarting dosing. The subject may be advised to premedicate with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. If NSAIDs are administered as a premedication, they should be given with food.

Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. If NSAIDs are administered as a premedication, they should be given with food.

Stopping Criteria:

If an NCI-CTCAE grade ≥3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

NUMBER OF SUBJECTS PLANNED:

Up to 100 subjects.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in this study must meet all of the following criteria:

- Must have completed participation in a previous rAvPAL-PEG study.
- Willing and able to provide written, signed informed consent, or, in the case of participants under the age of 18, provide written assent (if required) and written informed consent by a

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parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.

- Willing and able to comply with all study procedures.
- Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
- Sexually active subjects must be willing to use two acceptable methods of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
- Maintained a stable diet.
- In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
- Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
- Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
- A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's medical monitor. However, subjects who discontinued from study drug early due to a reaction are not eligible to enroll into this study.

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- Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
- Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).
- Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
- Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
- Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
- Creatinine > 1.5 times the upper limit of normal.

INVESTIGATIONAL PRODUCT, DOSE, ROUTE AND REGIMEN:

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or:

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ±0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

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Or:

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Fixed Dosing

Subjects who have demonstrated efficacy (ie, blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week).

DURATION OF TREATMENT:

Approximately 119 months

REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

None

CRITERIA FOR EVALUATION:

Efficacy:

Blood Phe concentrations will be measured.

Immunogenicity:

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The presence of antibodies (anti-PAL immunoglobulin G [IgG], anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies, anti-rAvPAL IgE, and anti-PEG-PAL IgE) will be assessed.

Safety:

Safety will be evaluated on the incidence of adverse events (AEs) and clinically significant changes in vital signs, ECG results, X-ray results, and laboratory test results.

Pharmacokinetic:

Plasma concentrations of rAvPAL-PEG will be measured for subjects participating in the Substudy.

STATISTICAL METHODS:

Sample Size:

Subjects who participated in a previous rAvPAL-PEG study may be enrolled into this study.
No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

Safety Analysis:

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF). The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by-subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics for vital signs, physical examination results, ECG results, X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

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Efficacy Analysis:

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the Statistical Analysis Plan [SAP]) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

Exploratory Analysis:

Details regarding exploratory analyses will be provided in the SAP.

Substudy Analysis:

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored.

Pharmacokinetic Analysis:

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F).

3 TABLE OF CONTENTS

LIST OF TABLES

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}7\ days}$	area under the plasma concentration-time curve from time zero on Day 1 to Day 8
BUN	blood urea nitrogen
°C	degree Celsius
C_3	complement 3
C_4	complement 4
CBC	complete blood count
CD	Compact disk
CFR	Code of Federal Regulations
CH50	total hemolytic complement
C_{max}	maximum plasma concentration
CNS	central nervous system
CO_2	carbon dioxide
CRA	Clinical Research Associate
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
DBP	diastolic blood pressure
DMC	Data Monitoring Committee
ECG	electrocardiogram
ETV	Early Termination Visit
FDA	Food and Drug Administration
F/U	follow-up
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GI	gastrointestinal

IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	identification
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeter of mercury
NAb	neutralizing antibodies
NCI	National Cancer Institute
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NOAEL	no observable adverse effect level
NSAID	non-steroidal anti-inflammatory medication
OTC	over the counter
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
Phe	phenylalanine
PI	Principal Investigator
PK	pharmacokinetics
PKU	phenylketonuria
rAvPAL-PEG	recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SC	subcutaneous

SD	standard deviation
SDV	source data verified
SGOT	serum glutamic oxalo-acetic transaminase
SGPT	serum glutamic pyruvate transaminase
US	United States
WBC	white blood cell

Definition of Terms:

Investigational Product (IP):

"A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from E6 ICH)

The terms "IP" and "study drug" may be used interchangeably in the protocol.

5 ETHICS

5.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) is properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) requirements, and applicable laws and local regulations. A copy of the confirmation from the IRB will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB confirming unconditional approval of the protocol, the ICF and all subject recruitment materials are obtained in writing by the PI and copies are received at BioMarin or its designee. The approval document should refer to the study by protocol title and BioMarin protocol number (if possible), identify the documents reviewed, and include the date of the review and approval. BioMarin will ensure that the appropriate reports on the progress of the study were made to the IRB and BioMarin by the PI in accordance with applicable governmental regulations.

5.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that address 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

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject, or his or her legally authorized representative will

provide written, informed consent before any study-related tests or evaluations are performed.

5.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, E6 ICH (GCP Guideline, Section 4.8) and 21 CFR Part 50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to BioMarin for approval prior to submission to the IRB. BioMarin and the IRB must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the clinical site must also be received by BioMarin or its designee prior to the delivery of study drug.

Subjects under the age of 18 years will provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) will provide written informed consent for such subjects. The Investigator will provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and will maintain the original in the subject's record file.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to BioMarin or its designee a fully executed and signed Form FDA (Food and Drug Administration) 1572 and a Financial Disclosure Form. All sub-Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub-Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of BioMarin. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. BioMarin's Pharmacovigilance Department (or designee) will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required. Some study assessments, including administration of study drug, may be performed at the subject's home. All assessments performed for this study will be administered by clinic staff or other qualified and trained study personnel.

Anti-rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase polyethylene glycol) immunoglobulin E (IgE) antibody analysis will be performed by a vendor; other antibody analysis will be performed by BioMarin. Clinical laboratory tests, including serum pregnancy tests (if applicable), will be analyzed and processed by a central laboratory, with the exception of erythrocyte sedimentation rate (ESR), and urine pregnancy tests (if applicable), which will be analyzed by a local laboratory. A central laboratory will also be used to perform analysis of blood phenylalanine (Phe) concentrations and urinalysis. Pharmacokinetic (PK) analysis will be performed by BioMarin.

Prior to database lock in multicenter studies, a Coordinating Investigator will be identified who will read the clinical study report and confirm that it accurately describes the conduct and results of the study, to the best of his or her knowledge. The Coordinating Investigator will be chosen on the basis of active participation in the study, ability to interpret data, and willingness to review and sign the report in a specified timeframe.

7 INTRODUCTION

A comprehensive review of PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase (rAvPAL-PEG) is contained in the IB supplied by BioMarin; the Investigator should review this document prior to initiating this study.

7.1 Nonclinical Studies

The nonclinical program for rAvPAL-PEG has characterized the pharmacology, pharmacokinetics (PK), and toxicology of rAvPAL-PEG using the intended clinical SC route of administration. Pharmacology was assessed in the BTBR *Pah*^{enu2} (ENU2) mouse model of PKU. The ENU2 mouse model exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM) and hypopigmentation. Following weekly doses of rAvPAL-PEG administered to male ENU2 mice, plasma Phe concentrations decreased from approximately 2000 μM to < 200 μM after 2 dose administrations. An attenuated reduction of blood Phe was seen between Week 3 and Week 7 of dosing. After 7 weekly administrations of rAvPAL-PEG, pharmacological activity returned as evidenced by stabilized concentrations of plasma Phe < 200 μM over the entire week, which continued for a total of 15 weeks.

In pharmacodynamic studies in the BTBR Pahenu2 (ENU2) mouse model administered 80 mg/kg/week rAvPEG-PAL, two dose interruption assessments were performed: a 2-4 week dose interruption and an 11-week dose interruption. Following both dose interruption assessments, the animals re-started rAvPAL-PEG at a dose of 80 mg/kg/week. No attenuated Phe response was observed when rAvPAL-PEG injections were temporarily stopped for 2-4 weeks, and there was an immediate return to stable blood Phe reductions. A similar interim attenuated response was observed with the longer dose interruption of approximately 11 weeks. In both of these assessments, no safety issues were noted upon re-starting dosing with rAvPAL-PEG (Lapis, 2007, ASHG 57th Annual Meeting).

Safety pharmacology studies were conducted in rats and cynomolgus monkeys. No respiratory or central nervous system (CNS) effects were seen with SC administration of rAvPAL-PEG. No changes in cardiovascular (CV) parameters, including electrocardiogram (ECG) waveforms, were observed in telemetry-instrumented monkeys assessed for the CV effects of rAvPAL-PEG administered subcutaneously.

Pharmacokinetic parameters were evaluated in single-dose and repeat-dose studies in rats and cynomolgus monkeys. The elimination half-life ($t_{1/2}$) values of rAvPAL-PEG administered to normal rats at SC doses of 10, 25, and 250 mg/kg were 47, 33, and 44 hours, respectively,

and exhibited modest area under the plasma concentration-time curve (AUC)-dose. In the cynomolgus monkey after SC administration of rAvPAL-PEG, the $t_{1/2}$ was 65 hours at 4.0 mg/kg, 71 hours at 12 mg/kg, and could not be determined at 60 mg/kg (the highest dose). Qualitatively, the kinetics of rAvPAL-PEG in the rat and monkey appear to have similar characteristics. The following findings were noted: (1) a 1-compartment model with first-order absorption appears to describe the plasma profiles of rAvPAL-PEG after single-dose administration, (2) absorption is slow and there is a long absorption period after SC administration to maximum observed plasma concentration (C_{max}), along with a long $t_{1/2}$, (3) elimination is slow and monophasic, (4) there does not appear to be a gender difference, and (5) the AUC and the C_{max} are roughly linearly proportional.

Toxicology of rAvPAL-PEG was assessed in single-dose and repeat-dose studies in normal rats and cynomolgus monkeys. In the single-dose rat study, decreased body weight gain and Kupffer cell vacuolization at the high SC dose of 250 mg/kg was observed; however, the Kupffer cell finding was not seen in the single-dose study in cynomolgus monkeys. In a 26-week repeat-dose toxicity and toxicokinetic (TK) rat study, decreased body weight gain, decreased food consumption, vacuolation/hypertrophy of the renal tubule cells, and vacuolation of the histiocytic cells were observed in rats administered 25 mg/kg/dose, twice weekly. In a 39-week repeat-dose study in monkeys, arterial inflammation, perivascular cellular infiltrates in the SC injection sites, thymic atrophy, and increased lymphoid nodules in bone marrow of the sternum were observed in monkeys given ≥ 3 mg/kg/dose, twice weekly. Of these findings, only the arterial inflammation was deemed adverse and this finding was completely reversed after 13-weeks of recovery. The no-observed-adverse-effect-level (NOAEL) of rAvPAL-PEG when administered subcutaneously twice weekly for 26 weeks and 39 weeks in the rat and monkey, respectively, was 1.0 mg/kg. These NOAELs were based on histological findings of vacuolation/hypertrophy of the renal tubule cells in the rat and arterial inflammation findings in the monkey.

7.2 Previous Clinical Studies

The rAvPAL-PEG clinical development program has been designed to demonstrate the safety and efficacy of rAvPAL-PEG in reducing blood Phe concentrations in PKU subjects who do not respond to treatment with Kuvan® or are not compliant with Kuvan® treatment. This study is an extension of the ongoing human clinical studies with rAvPAL-PEG (Study PAL-002, Study PAL-004, and Study 165-205) based on data from the completed clinical study, PAL-001, and clinical experience from this ongoing study and the other ongoing Phase 2 studies, PAL-002 and PAL-004.

7.2.1 Phase 1 Study, PAL-001

The first-in-human trial using rAvPAL-PEG, Study PAL-001, was a Phase 1, open-label, dose-escalation study in PKU subjects in the US. The primary objective was to assess the safety and tolerability of single SC injections of rAvPAL-PEG. Secondary objectives were to evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood Phe concentrations.

The effectiveness of rAvPAL-PEG in decreasing Phe concentrations in nonclinical studies of ENU2 mice was consistent with what was observed in PAL-001. PAL-001 enrolled 25 PKU subjects with a mean (standard deviation; SD) baseline blood Phe concentration of 1310 µmol/L (405). The 25 subjects who enrolled into the study were assigned to receive a single administration of rAvPAL-PEG at 1 of 5 dose levels: 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg. Five subjects each received 1 of the 5 dose levels of rAvPAL-PEG.

Key safety, efficacy, and PK results of Study PAL-001 are summarized as follows:

- rAvPAL-PEG was well tolerated and had a clinically significant blood-lowering effect on Phe concentration when administered at a dose of 0.1 mg/kg.
- For the 5 subjects who were administered 0.1 mg/kg rAvPAL-PEG, clinically significant reductions in blood Phe level were observed, with the most clinically significant decreases from baseline values to Day 7, ranging from 33.8% to 97.3% (mean of 58.12%). The mean baseline blood Phe level for these 5 subjects was 1113 µmol/L.
- Overall, no clinically significant reductions in blood Phe level were observed in subjects who were administered rAvPAL-PEG at the lower doses of 0.001, 0.003, 0.01, and 0.03 mg/kg.
- For subjects who were administered rAvPAL-PEG at the 3 higher dose levels (0.01, 0.03, and 0.1 mg/kg) and who had measurable rAvPAL-PEG concentration levels, the calculated PK parameters were a mean T_{max} of 89-106 hours, a $t_{1/2}$ of 46-120 hours, and an AUC_{0-t}, and C_{max} that were proportional with dose. Drug exposure was slightly increased with increased dose.

7.2.2 Phase 2 Studies PAL-002, PAL-004, and 165-205

Based on results from the Phase 1, single-dose study, the Phase 2 studies have been designed to evaluate rAvPAL-PEG at various doses and dosing regimens to safely achieve and maintain blood Phe reductions in subjects with PKU. Currently, there are three ongoing Phase 2 studies (PAL-002, PAL-004, 165-205); an overview of each study is presented in Section 7.2.2.1 (PAL-002), Section 7.2.2.2 (PAL-004), and Section 7.2.2.3 (165-205).

7.2.2.1 Study PAL-002

Study PAL-002 (A Phase 2, Open-Label, Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects With Phenylketonuria) was initiated in September 2009 and has completed enrollment. As of 10 September 2012, 40 subjects have been enrolled and 37 have completed the study. PAL-002 enrolled 11 previously exposed subjects from the single-dose study, PAL-001, and 29 subjects who were naïve to rAvPAL-PEG. Thirty-three of the 37 subjects who completed PAL-002 enrolled into this open-label extension study (PAL-003). Two subjects discontinued from the study due to lost to follow-up, relocation, or other reasons, and 1 subject discontinued from the study due to an AE (skin reaction).

The primary objective of this study was to evaluate the effect of multiple doses of rAvPAL-PEG (ranging from 0.001 mg/kg/week to 1.0 mg/kg/week) on blood Phe concentrations in subjects with PKU with up to 16 weeks of treatment. The secondary objectives of the study were to evaluate the safety and tolerability of SC injections of multiple doses of rAvPAL-PEG, to evaluate the immune response to rAvPAL-PEG, and to evaluate the PK profile of rAvPAL-PEG in subjects with PKU.

The PAL-002 study design consists of two parts. In Part 1, rAvPAL-PEG was administered as a once weekly fixed, low-dose induction regimen for 8 weeks. In Part 2, the rAvPAL-PEG dose was titrated upwards; subjects received adjustable dose increases for up to 8 weeks to achieve a target blood Phe concentration of 600 µmol/L. The doses and dosing schedules were revised with a series of protocol amendments to incorporate the information gained during conduct of this early, open-label, multiple-dose study.

A wide range of doses was planned for the PAL-002 study, beginning with doses as low as 0.001mg/kg/week. However, no substantial reductions in blood Phe level were observed in the majority of subjects who were administered rAvPAL-PEG in the initial four cohorts at doses of 0.001, 0.003, 0.01, and 0.03 mg/kg administered once per week. The absence of appreciable Phe reduction after 16 weeks of treatment in this range of doses led to a decision to amend the study protocol to include a cohort with a higher starting dose of 0.1 mg/kg/week, which had been previously shown to decrease blood Phe levels to approximately 600 µmol/L in the single-dose, Phase 1 study (PAL-001). After the first 2 weeks of dosing at 0.1 mg/kg/week, transient Phe reduction was observed in this subset of subjects. However, this dosing regimen was accompanied by mild to moderate hypersensitivity reactions primarily after the second weekly dose, suggesting that additional exploration of dosing regimens would be required.

In summary, preliminary results from PAL-002 demonstrated that doses below 0.1 g/kg administered once per week were not effective in reducing Phe levels. While transient reduction of Phe was apparent at doses of at least 0.1 mg/kg given once per week, this dose regimen was associated with a high incidence of systemic hypersensitivity reactions, typically following the second weekly dose. Further escalation above this dose of 0.1 mg/kg/week would be required to sustain the effect on Phe levels over time. Increased doses would require spreading dose administration over several days per week, as once-weekly administration of higher doses would not be practical due to the large volume of study drug. Therefore, additional dosing regimens would need to be explored in a subsequent study (PAL-004).

Once subjects completed Study PAL-002, they were eligible to enroll into this open-label extension study, PAL-003 to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.2 Study PAL-004

PAL-004 (An Open-Label Study to Evaluate the Safety, Tolerability, and Efficacy of Subcutaneous Dose Levels of rAvPAL-PEG Administered Daily in Subjects With Phenylketonuria) was initiated in April 2011 and has completed enrollment. As of 10 September 2012, 16 subjects have been enrolled, 15 subjects have completed the study, and 15 subjects have enrolled into this study (PAL-003). One subject withdrew consent from continued participation prior to study completion. All subjects enrolled in this study were naïve to rAvPAL-PEG exposure.

The objective of this study was to determine if daily administration (defined as 5days/week) of rAvPAL-PEG at dose levels of 0.06, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/kg/day was safe and effective in reducing and maintaining blood Phe concentrations to 600 µmol/L in subjects with PKU. The starting dose was 0.4 mg/kg, administered 5 days/week. A total of 12 subjects were added in sequence and were started on doses of 0.4, 0.2, 0.1, 0.06 mg, or 0.001/kg/day based on incoming safety data. Overall, subjects in PAL-004 who initiated treatment at higher daily doses of 0.1 to 0.4 mg/kg/day achieved immediate and substantial reduction of blood Phe levels, but dosing had to be temporarily reduced or interrupted due to the onset of systemic hypersensitivity reactions at approximately Day 10 of dosing. Phe reductions did not persist in the setting of temporary dose reduction or interruption, but Phe levels generally improved if higher dose levels were reinstated.

To prevent the onset of hypersensitivity reactions that were temporally associated with the onset of anti-drug IgM responses, an additional dosing strategy was assessed in PAL-004;

4 subjects initiated dosing at a low dose of 0.001mg/kg/day or 0.06mg/kg/day for 5 consecutive days and then dosing was suspended for 2 weeks. Dosing was restarted on Day 21 at the initial dose followed by dose titration to target Phe levels. Subjects administered this dosing regimen had similar efficacy, but the incidence of hypersensitivity reactions appeared to be similar to that of subjects who did not have the planned dose interruption, suggesting little advantage with this alternate induction regimen.

Experience from PAL-004 indicated that initiating daily rAvPAL-PEG dosing with relatively high daily doses was not sustainable due to the onset of hypersensitivity reactions that occurred approximately 9 to 12 days after the start of administration, nor was the alternate dosing regimen (2 week drug holiday) well tolerated or effective in Phe reduction. In addition, the daily treatment regimen did not reduce the time to achieve target blood Phe levels seen in previous studies. This study indicated that to improve tolerability, rAvPAL-PEG should be initiated with an induction period at doses substantially lower than 0.4mg/kg, followed by upward dose titration toward target Phe levels.

Once subjects completed Study PAL-004, they were eligible to enroll into this open-label extension study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week.

7.2.2.3 Study 165-205

Study 165-205 (A Phase 2, Multi-Center, Open-Label, Dose-Finding Study to Evaluate Safety, Efficacy, and Tolerability of Subcutaneously Administered rAvPAL-PEG in Subjects With PKU for 24 Weeks) was initiated in May 2012. Data from ongoing Studies PAL-002, PAL-004, and PAL-003 suggested that a 4-week treatment course with weekly dosing at a fixed low dose (induction), followed by a weekly 2-fold, upward titration of rAvPAL-PEG to approximately 10-fold higher than the initiation dose (maximum of 375 mg/week) is effective and well tolerated. The objective of this study was to further assess this dosing regimen. Two rAvPAL-PEG dosing regimens were to be explored in this study: weekly low-dose induction (2.5 mg/week for 4 to 8 weeks), followed by a period of upward dose titration towards a target Phe level (600 µmol/L) followed by maintenance dosing at that level through the 24 week study duration (Group 1). The other dosing regimen planned for this study (Group 2) involved administration of a single bolus dose of 8 mg, followed by a treatment holiday of at least 3 weeks duration followed by resumption and escalation of study drug administration following a pattern similar to that employed in Group 1. A total of 24 subjects were enrolled into 165-205. In addition to confirming an effective, well-tolerated dose regimen, this study incorporated non-weight-based dosing, starting with dose

administration once per week at a low dose (2.5mg) with gradual escalation and conversion of dose administration to 5x/week dosing for chronic maintenance therapy.

Once subjects have completed Study 165-205, they are eligible to enroll into this study to continue to receive rAvPAL-PEG up to a maximum dose of 5.0 mg/kg/week or 375 mg/week, or into the Phase 3 study, 165-302.

7.3 Study Rationale

PKU is an autosomal, recessive, inherited disease characterized by deficiency in the enzyme PAH. Approximately 1 in every 10,000 infants in North America is born with PKU (Scriver, 2001, McGraw-Hill). Left untreated, PAH deficiency is associated with an abnormally elevated concentration of Phe, which is toxic to the brain (Kaufman, 1989, J Pediatr.) and can result in a variety of neuropathologic conditions, including mental retardation, microcephaly, delayed speech, seizures, and behavioral abnormalities (Scriver, 2001, McGraw-Hill). The brains of untreated patients with PKU show evidence of hypomyelination and gliosis, arrest in cerebral development, and, in some cases, white matter degeneration (Huttenlocher, 2000, Eur.J Pediatr.). Elevated Phe during fetal development leads to severe mental retardation and can cause microcephaly, growth retardation, and congenital heart disease (Guttler, 2003, Pediatrics), (Koch, 2003, Pediatrics), (Lee, 2003, Pediatrics), (Levy, 2003, Pediatrics), (Matalon, 2003, Pediatrics), (Rouse, 2004, J.Pediatr.).

For a subset of patients with residual enzyme activity, treatment with Kuvan® is an option. For all other patients, current management of PKU involves adherence to low-Phe medicinal diet formulas and dietary restriction of Phe-containing foods. Implementation of a Phe-restricted diet in infancy significantly reduces blood Phe concentrations and prevents many of the neuropathologic manifestations of PKU, including severe mental retardation. However, compliance with this restrictive diet can be difficult for older children, adolescents, and adults, and adherence may result in nutritional deficiencies due to the limitations of natural protein sources (Fisch, 2000, Eur.J.Pediatr.), (Walter, 2002, Lancet).

The need exists for a treatment that will help individuals with PKU safely achieve lifelong, reliable control of blood phenylalanine (Phe) concentrations and improve functional outcomes. rAvPAL-PEG is a potential substitute for the phenylalanine hydroxylase (PAH) enzyme, which is defective in individuals with PKU. rAvPAL-PEG may control blood Phe concentrations, which may have additional clinical benefits.

Blood Phe levels depend not only on the functional presence of the endogenous enzyme PAH but also on the dietary intake of Phe. It is important to understand the role of diet on blood Phe levels concurrent with the administration of rAvPAL-PEG treatment. Therefore, an

exploratory objective has been added to this study to assess the relationship of diet, rAvPAL-PEG administration, and blood Phe level. Changes to subject diet are not allowed in this study unless per the Investigator. Diet will be monitored using a diet diary.

A pharmacokinetic (PK) substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Single-dose PK data has been collected in the Phase 1 study, PAL-001. However, there is little multiple-dose PK data or data for when subjects have reached and maintained a stable dosing regimen. With the PK substudy, multiple-dose PK data will be collected for subjects who have already achieved Phe reduction to within the protocol-defined target range.

This study is an extension of previous rAvPAL-PEG Phase 2 studies. Administration of rAvPAL-PEG will be continued to assess the long-term safety and efficacy of rAvPAL-PEG in PKU subjects.

7.4 Summary of Overall Risks and Benefits

It is not expected that data from previous rAvPAL-PEG studies will change the assumption that rAvPAL-PEG has a toxicity profile similar to other recombinant PEGylated non-human enzymes currently in use. The toxicities of these drugs usually fall into 2 main categories: toxicity due to exposure to PEG, and toxicity due to immunologic sensitization. In addition, exaggerated pharmacological effects resulting in very low Phe concentrations could be observed. Other adverse events (AEs) that could occur with rAvPAL-PEG include injection-site reactions and other general symptoms.

7.4.1 Toxicity Due to Exposure to Polyethylene Glycol (PEG)

Acute exposure to high doses of PEG can result in renal toxicity. There have been a few reports of PEG-related AEs in humans; cumulative intravenous (IV) PEG doses of 121 to 240 g caused acute renal tubular necrosis, oliguria, and azotemia. The anticipated PEG exposure associated with the expected rAvPAL-PEG dose range (6 g per week) is at least 80-fold lower than the reported toxic doses and comparable to exposures to PEG in widely used medicines and other compounds that are administered by the IV, oral, rectal, and topical routes (Webster, 2007, Drug Metab Dispos.). No indication of PEG-related toxicity at the ultrastructural level was observed in monkeys given a single dose of up to 60 mg/kg rAvPAL-PEG SC. In rats, both renal tubule vacuolation and histiocytic cell vacuoles have been assocated with PEG-related catabolism and administration of other PEGylated proteins (refer to Section 7.1).

Based on phase 3 data, as of 4MAR2016, there have been no reported events of glomerulonephritis or renal failure reported with pegvaliase administration. Of the 10 subjects identified with UAC ratios ≥ 3 mg/mmol on 3 or more consecutive measurements, values were generally in the microalbuminuria range and none had concurrent sustained hematuria. Seven of these 10 subjects had microalbuminuria at baseline prior to pegvaliase exposure, of which 2 events resolved while on pegvaliase treatment and 4 events did not progress while subjects remained on pegvaliase. One of these 7 subjects with baseline microalbuminuria (3.62 mg/mmol at baseline) had an increase in their UAC ratio during the study, which was more apparent after dose titration to 60 mg of pegvaliase (remaining in microalbuminuria range, with a most recent value of 20.36 mg/mmol), however evaluating relationship to pegvaliase treatment is confounded by a medical history of obesity, hypertension, and new onset pre-diabetes during the Phase 3 study that are conditions also associated with development of microalbuminuria. The subject continues on pegvaliase treatment with continued safety monitoring.

Three of the 10 subjects developed new onset microalbuminuria for at least 3 consecutive measurements during the Phase 3 study, one of which resolved while continuing on pegvaliase treatment and 1 subject continues to have fluctuating low level microalbuminuria with intermittent values in the normal range. One subject discontinued from the Phase 3 study after a few intermittent elevations into the microalbuminuria range. The subject had a maximum UAC ratio of 175 mg/g on Day 8 on the study; at the time of study and study drug discontinuation (Day 36), UAC ratio was within the normal range at 19 mg/g. The final study visit recorded for this subject (Day 67) had a UAC ratio of 17 mg/g. This subject reported a single concurrent grade 1 event of urticaria, which resolved in 2 days, and no other reported events suggestive of immune complex disease. None of the subjects described above have developed sustained elevations in serum creatinine or concurrent sustained hematuria.

To monitor for renal toxicity, subjects participating in this study will undergo urinalysis, will be assessed for urine albumin/creatinine ratio, and will undergo blood chemistry tests to assess renal function.

7.4.2 Immunologic Response

The active drug substance in rAvPAL-PEG is a bacterial protein and elicits immune recognition and subsequent antibody responses. Antibody epitopes on the protein are expected to be rendered at least partially inaccessible by the extensive PEGylation on the drug (Gamez, 2007, Mol.Genet.Metab). In vivo and in vitro experiments have shown that PEGylation of proteins and cells can attenuate the immunological response (Scott, 1997, Proc.Natl.Acad.Sci.U.S.A), (Chen, 2001, BioDrugs.). PEGylation has been effective in

reducing anti-rAvPAL antibody titers in the mouse model but does not eliminate antibody formation. Although PEGylation of rAvPAL likely reduces the antigenicity of the molecule, 100% of individuals exposed to rAvPAL-PEG develop antibodies against rAvPAL.

Drug-specific immune responses can range from clinical irrelevance to causing serious hypersensitivity AEs (such as anaphylaxis) and may reduce efficacy due to antibody-mediated drug clearance or antibody neutralization of enzymatic activity. Historically, PEG was thought to be nonimmunogenic (Davis, 1981, Clin.Exp.Immunol.), (Harris, 2003, Nat.Rev.Drug Discov.); however, repeated studies have found that subcutaneous (SC) and intravenous exposure to PEG can induce anti-PEG antibodies (Harris, 2003, Nat.Rev.Drug Discov.), (Richter, 1983, Int.Arch.Allergy Appl.Immunol.). In some instances, development of such antibodies did not result in significant clinical effects in humans (Richter, 1984, Int.Arch.Allergy Appl.Immunol.). However, PEG antibody responses were associated with hypersensitivity reactions to PEGylated liposomes (Judge, 2006, Mol.Ther.). Anti-PEG antibodies have also been associated with nonresponsiveness against therapy (Armstrong, 2007, Cancer), (Ganson, 2006, Arthritis Res.Ther.).

Subjects participating in the Phase 2 clinical studies have been monitored closely for anti-drug immunogenicity (anti-rAvPAL immunoglobulin G [IgG], anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, neutralizing antibodies that inhibit PAL enzymatic activity, anti-rAvPAL IgE, and anti-rAvPAL-PEG IgE), complements, C-reactive protein (CRP), ESR, and complete chemistry. All subjects treated with rAvPAL-PEG in the Phase 2 clinical trials developed antibodies against the PAL protein. Anti-rAvPAL IgM antibodies were first detected within 2-3 weeks of treatment initiation and anti-rAvPAL IgG antibodies were detected within 2-3 months. The anti-rAvPAL IgG response generally peaked by 3-5 months and was sustained in all subjects. Neutralizing antibodies (NAb) capable of inhibiting enzymatic activity were detected in a minority of subjects 2-3 months after treatment initiation. The timing of the NAb response suggests these antibodies may be a component of the anti-rAvPAL IgG response. It is expected that the centralized location of the PAL active site, within the tetrameric structure, makes it difficult for antibodies to bind and inhibit enzymatic activity. In addition, anti-PAL antibody binding is likely sterically hindered by the extensive PEGylation. The majority of treated subjects also developed a transient anti-PEG antibody response. The anti-PEG response was comprised of IgM and IgG isotypes. In general, anti-PEG antibodies were no longer detected after 5-6 months of treatment. This type of transient antibody response is typical of T cell-independent type 2 (TI-2) responses.

The majority of immune-mediated AEs experienced in subjects treated with rAvPAL-PEG in the Phase 2 studies are believed to be the result of antibody-mediated hypersensitivity. The

primary immune mediator is thought to be IgM immune complexes, as evidenced by the timing of onset prior to IgG development and the reduced occurrence over time. IgE positive test results have not been associated with hypersensitivity in any of the subjects thus far, and the majority of subjects have been safely rechallenged with rAvPAL-PEG following reactions. IgM immune complexes are known to be efficient activators of the classical complement pathway and can lead to the accumulation of complement component anaphylatoxins (C3a, C4a, C5a) that can induce hypersensitivity symptoms. Although IgG immune complexes can also activate the classical complement pathway, they do so with less efficiency than IgM. This is due, in part, to the requirement for multiple Fc regions in close proximity to activate the complement cascade, meaning that multiple monomeric IgG Abs are required to bind, whereas only one pentameric IgM Ab is needed to bind to activate complement. Also, in the case of rAvPAL-PEG, binding of anti-PAL IgG antibodies is likely compromised due to epitope masking by the PEGylation, which may reduce IgG-mediated complement activation *in vivo*. This complex dynamic between combined isotype-specific Ab responses and epitope accessibility is thought to be a major factor dictating the need for an induction and titration dose regimen in the treatment of rAvPAL-PEG to be implemented in this study.

7.4.3 Management of Hypersensitivity Reactions

Hypersensitivity reactions to the SC injection of rAvPAL-PEG may be mild, moderate**,** or severe in intensity. Reactions may include pruritus, rash, shortness of breath, hypotension, or anaphylaxis. Clinical assessments should be conducted for a minimum of 30 minutes post-injection. Longer observations may be required at the discretion of the PI.
The responsible physician should use all appropriate measures for the treatment of hypersensitivity reactions. Because of the potential for anaphylaxis, equipment for emergency resuscitation (including epinephrine) should be within easy access during and after the rAvPAL-PEG injection. Subject should be pre-medicated with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. If non-steroidal anti-inflammatory medication (NSAIDs) is administered as a premedication, it should be given with food. Subjects who qualify for self-administration of study drug are trained to recognize a serious hypersensitivity AEs and how to respond. Additionally, subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (Section 9.4). Refer to Section 9.1.1.3 for instructions regarding resumption of study drug following resolution of symptoms consistent with a clinical diagnosis of anaphylaxis per National Institute of Allergy and Infectious

Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria (Sampson, 2006, J.Allergy Clin.Immunol.).

In the event of a hypersensitivity reaction, subjects may be asked to see an allergist/immunologist and/or provide up to 3 additional blood samples for immunology studies and complement testing. This additional testing may occur up to 6 months following the final study visit.

The following measures are recommended for the treatment of hypersensitivity reaction symptoms:

- Maintain the airway.
- Vital sign check.
- Administration of oral or IV diphenhydramine.
- Administration of epinephrine if appropriate.
- Administration of oral or IV glucocorticoids.
- Administration of oxygen and IV fluids.
- Administration of additional symptomatic treatment.
- Transfer to an acute care facility of the study center.

In addition, clinical judgment must be used in determining the appropriateness of corticosteroid administration. An allergy and/or immunology consultation should be sought if necessary. Detailed instructions for the management of hypersensitivity reactions are provided in the Study Reference Manual.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the effect of long-term administration of multiple doses of SC injections of rAvPAL-PEG on blood Phe concentrations in subjects with PKU.

The secondary objectives of the study are:

- To evaluate the safety and tolerability of long-term administration of subcutaneous (SC) injections of rAvPAL-PEG in subjects with PKU.
- To evaluate the immune response to long-term administration of SC injections of rAvPAL-PEG in subjects with PKU.

The exploratory objective of the study is as follows:

- To evaluate the long-term relationship of diet and change in blood Phe concentration following administration with rAvPAL-PEG in subjects with PKU.

The Substudy objective is as follows:

- To evaluate steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe concentration and for whom no further dose modifications are planned.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is a long-term extension of rAvPAL-PEG Phase 2 studies in up to 100 subjects with PKU. The doses are planned to be in the same range as those previously tested in the Phase 2 studies (starting at 0.001 through 5.0 mg/kg/week or 2.5 mg through 375 mg/week), provided no dose-limiting toxicity was observed in a previous rAvPAL-PEG studies. Subjects' doses will not exceed 375 mg/week.

Only subjects who completed a previous rAvPAL-PEG study will be enrolled into this study. Subjects enrolling into PAL-003 with no interruption in dosing from a previous rAvPAL-PEG study may either continue their previous dosing regimen or begin this study at a higher dose level. Subjects who have had dosing interruptions between the previous rAvPAL-PEG study and PAL-003 must have their starting dose determined per the sponsor's medical monitor. The first week of dosing will be administered in the clinic, and the subject must be observed for 30 minutes following administration. Subjects are given two epinephrine injectors and are instructed to carry one epinephrine injector with them at all times (refer to Section 9.4). A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing.

Diet should not be altered during the course of this study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. To monitor for changes in subject diet, a diet diary will be issued to subjects for completion and will be brought to clinic monthly for review with the clinical study staff.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects must meet a set of criteria to be considered appropriate for self-administration. Refer to Section 9.4 for the criteria for which subjects may qualify for self-administration. Additional information is provided in the Subject Self-Administration Training Materials. Additional training will be provided and demonstration of competency required when prefilled syringe drug product is introduced, every 24 weeks, and more often as needed.

Subjects will be evaluated for safety throughout the study. Toxicity will be measured according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.

In addition to the Sponsor, a Data Monitoring Committee (DMC) will monitor the safety of subjects in the study. The DMC is an independent committee that will act in an advisory capacity to the Sponsor.

PAL-003 is designed to evaluate long-term treatment of subjects who are continuing to take rAvPAL-PEG. Subjects'previous rAvPAL-PEG dosing will generally continue in PAL-003. In PAL-003, each subject's dose will be adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60-600 µmol/L. rAvPAL-PEG dose will be based on either a subject's weight or will be a fixed dose. Doses will be evaluated on an individual basis.

Evaluations, observations, and procedures will be conducted at selected time points as shown in Table 9.1.1 and Table 9.1.2. After the subject's blood Phe concentration has been controlled to within a target range (60-600 µmol/L), the subject may be asked to have additional blood draws for PK and blood Phe concentration analyses as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

A subject will continue in PAL-003 until one of the following occurs:

- The subject withdraws consent and discontinues from the study.
- The subject is discontinued from the study at the discretion of the Investigator or Sponsor.
- The subject has completed the study through the Week 476 visit.
- The study is terminated.
- The study drug receives marketing authorization.

Because the risks of taking BMN 165 during pregnancy and breastfeeding are unknown, subjects cannot take BMN 165 if they are trying to conceive, are pregnant, or are breastfeeding (refer to Section 9.3.2 for Exclusion Criteria). Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study must be temporarily discontinued from study drug for 4 weeks prior to trying to conceive. During that time, subjects must use two acceptable methods of contraception. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician. Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests. Subjects who are pregnant or are trying to conceive and have temporarily discontinued study drug should not perform the scheduled PK assessments. Male subjects who have impregnated a female partner may

re-start study drug after conception following the investigator's consultation with and approval by the medical monitor. Male subjects must return to the study-required contraception use (refer to Section 9.3.1 for Inclusion Criteria), which must include one barrier method, prior to restarting study drug. Female subjects who remain in the study after temporary discontinuation of study drug due to pregnancy may restart BMN 165 dosing after a confirmed negative urine pregnancy test result, the birth has been reported (or termination of the pregnancy), and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting BMN 165 dosing requires the investigator's prior consultation with and approval by the medical monitor. Female subjects must return to the study-required contraception use (refer to Section 9.3.1), which must include one barrier method, immediately after the birth (or termination of the pregnancy).

Subjects may withdraw from treatment with study drug at any time; however, subjects will be asked to continue to perform the study assessments until study completion. Subjects may also withdraw from study participation at any time. Because the completeness of the data affects the integrity, accuracy, and interpretation of study results, every effort should be made to retain subjects in the study and to have them complete the study assessments as scheduled as long as continued participation does not compromise subject safety.

A PK substudy has been added to evaluate the steady-state PK of rAvPAL-PEG in up to 6 subjects who have achieved and maintained target blood Phe for at least 2 consecutive measurements and for whom no further dose modifications are planned. Subjects enrolled into the substudy will have additional pre-dose PK sampling performed (refer to Table 9.1.2).

The Schedule of Events and PK substudy collection schedules are presented below in Table 9.1.1 and Table 9.1.2.

Table 9.1.1: Schedule of Events

Assessments and Events	Screening[a]	Treatment Period[b,c]			Self-Administration	Study Completion/Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Every 8 Weeks[b]			
		D 1	Starting at Week 2			4 weeks after final dose	
Informed consent	X						
Medical history, including allergy history, and demographics	X						
Physical examination[e]	X	X		X		X	
Vital signs[e]	X	X		X		X	X
Weight	X			X			
12-lead ECG	X					X	
Clinical laboratory tests[f]	X			X		X	X[g]
Complements C_3 and C_4[h]	X			X			X[g]
Sedimentation rate		X		X		X	
Chest x-ray	X			X (Week 48 visit only)		X	
Urine pregnancy test[i]	X	X		X		X	
Adverse events[j,k]	X	X	X	X	X	X	X
Weekly phone call to self admin participants only (to assess AEs, Inj site reactions, concomitant medications)			X		X		
Concomitant medications	X	X	X	X	X	X	X
Diet query	X	X		X		X	
3-Day diet diary[l]				X		X	
Serum antibodies[m]		X		X		X	X
Plasma Phe and plasma tyrosine[n]	X	X		X[m]		X	

Assessments and Events	Screening[a]	Treatment Period[b,c]			Self-Administration	Study Completion/Early Term. Visit	Unsched. Hyper. Reaction Visit.[d] Perform within 24 hours
		Week 1	Weekly Telephone Contacts	Every 8 Weeks[b]			
		D 1	Starting at Week 2			4 weeks after final dose	
Urine albumin/creatinine ratio [o]							X
Urine N-methyl histamine							X
Plasma PK sample[p]		X		X		X	
Administer study drug[q]		X		X	X		
Training (prefilled syringe)[r]				X Every 24 weeks only			
Skin biopsy (optional; affected and not affected area)							X
Serum tryptase level[s]							X

AE, adverse event; C_3, complement 3; C_4, complement 4; CH50, total hemolytic complement; CRP, C-reactive protein; D, day; ECG, electrocardiogram; eCRF, electronic Case Report Form; ETV, Early Termination Visit; F/U, Follow-up; Hyper, hypersensitivity; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IP, investigational product; PK, pharmacokinetics; Phe, phenylalanine; SAE, serious adverse event; Wk, week.

[a] A separate Screening visit for this study is required only if the time between completion of the previous rAvPAL-PEG study and enrollment into PAL-003 is greater than 28 days.

[b] Visits must be performed in the clinic.

[c] On days that a dose is given, all procedures should be performed predose, except where noted.

[d] Refer to Section 9.1.1 and Section 12.3.6.

[e] Complete physical examinations to include the evaluation of all major body systems. Height will be measured at Screening only.

[f] Clinical laboratory tests to include spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis. Urine microscopy will be performed if any urinalysis results are positive for hematuria. It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Refer to Table 9.7.6.1.

[g] Subjects who have a National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade ≥ 3 hypersensitivity AE should be assessed for CRP, CH50, C1, C3, and C4 within 24 hours of the reaction.

[h] Complement C3 and C4 will be collected at the Screening Visit and every 8 weeks. Additional complement testing may be performed as needed to resolve previous abnormal test results.

[i] If positive or equivocal, perform serum pregnancy test. Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician. Also refer to Section 9.1.

[j] If any injection site reaction is observed, the corresponding adverse event data must specify injection location (identify current vs. previous visit's injection, eg, right arm vs. left arm). If there is a skin reaction that lasts ≥14 days, the Skin Reaction eCRF should be completed. Subjects who experience an injection-site skin reaction that lasts ≥14 days should be referred to a dermatologist for consultation and a skin biopsy. It is recommended that a photograph of the skin reaction be taken by the subject or the site to help assess the event; photographs may be collected by the sponsor.

[k] The reporting period for SAEs begins when informed consent is obtained and continues through 4 weeks after last dose or completion of study participation, whichever occurs last. The reporting period for nonserious AEs is from the first administration of study drug through 4 weeks after last dose or the Study Completion Visit/Early Termination Visit, whichever occurs last. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe.

[l] It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.

[m] For unscheduled hypersensitivity reaction visit, draw serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only; sampling must be performed > 8 hours after event onset and before the next dose of study drug.

[n] Samples should be drawn at least 2.5 hours after a meal. At the Investigator's discretion, blood Phe may be collected more often should more frequent monitoring of blood Phe levels be clinically warranted.

[o] It is recommended that urine samples are obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at baseline. Subjects who had elevated results at baseline followed by a confirmed subsequent increase of 100-200 mg/g from baseline should also be referred to a nephrologist for consultation.

[po] Sampling should be performed predose. Subjects who are pregnant or are trying to conceive and have temporarily discontinued study drug should not perform the scheduled PK assessments.

[q] Dosing is up to 5.0 mg/kg/week or 375 mg/week, including subjects who receive more than 1 dose/week. Every effort should be made to adhere to the study drug administration regimen. If a subject misses more than 2 scheduled doses of study drug, both dose level and dose frequency may be re-evaluated.

[r] Initial training on the use of prefilled syringe occurs at the next monthly visit and each subsequent day after transition until (in clinic or by HHRN) it has been determined the subject will switch to prefilled syringe. Once competency is documented, subjects can self-administer study drug using the prefilled syringe. Regular refresher prefilled syringe training occurs in the study clinic every 24 weeks thereafter.

[s] Mandatory for subjects who have a hypersensitivity AE. Perform within 24 hours of event onset.

Table 9.1.2: PK Substudy Dosing Regimens

Treatment Frequency	Plasma PK Sampling	Example
Subjects who are administered study drug once per week	Pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours when possible), and pre-dose of the next weekly dose	If dosed on Monday: Obtain PK sample pre-dose when possible – pre-dose Monday, Tuesday, Wednesday, Thursday, Friday, and pre-dose the following Monday. No further PK substudy draws.
Subjects who are administered study drug two or three times per week	Pre-dose and every 24 hours during the longest period between doses, when possible, and pre-dose of the next dose.	If dosed on Monday, Thursday, and Friday: Obtain PK sample every 24 hours during the longest period between doses: Pre-dose Monday, Tuesday, Wednesday, and pre-dose Thursday. No further PK substudy draws.
Subjects who are administered study drug four to seven times per week.	Pre-dose and every 12 hours during the longest period between doses, when possible, then pre-dose of the next dose.	If dosed Monday through Friday: Obtain PK sample every 12 hours during the longest period between doses (Friday to Monday): pre-dose Friday, 12 hours post-dose Friday, Saturday (24 and 36 hours post dose), Sunday (48 and 60 hours post-dose), and pre-dose the following Monday. No further PK substudy draws.

9.1.1 Response to Hypersensitivity Adverse Events

Subjects are evaluated for safety throughout the study and are trained to recognize potential hypersensitivity AEs (including anaphylaxis) and how to respond. Subjects are instructed to contact the investigator for any suspected hypersensitivity AE. The investigator may require further evaluation at the clinic. If a hypersensitivity AE (eg, injection-site reaction, rash, joint pain, itching) occurs, the subject may be advised to premedicate with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to subsequent rAvPAL-PEG doses. If NSAIDs are administered as a premedication, they should be given with food. Subjects who experience an injection-site skin reaction that lasts ≥14 days should be referred to a dermatologist for consultation and a skin biopsy.

Hypersensitivity AEs, including anaphylaxis, are expected with rAvPAL-PEG administration. rAvPAL-PEG dosing in response to a suspected hypersensitivity AE may be modified or halted depending on the severity of the event and suspected rAvPAL-PEG causality (Section 9.1.1.3). Severity for hypersensitivity AEs will be per NCI-CTCAE grades.

9.1.1.1 Individual Stopping Criteria

Subjects who have an NCI CTCAE grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug.

9.1.1.2 Dosing in Response to Hypersensitivity Adverse Events

Dosing in response to a hypersensitivity AE depends on the NCI-CTCAE grade and suspected relationship to rAvPAL-PEG. Dosing instructions are presented in Table 9.1.1.2.1 and are regardless of previous occurrence:

Table 9.1.1.2.1: Dosing Instructions in Response to Hypersensitivity Adverse Events

NCI-CTCAE Grade[a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria [d]	HRV Assessment[e]
		Maintain[b]	Reduce[c]	Interrupt[c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No	X	(X) Optional	(X) Optional		Investigator discretion
3	Yes	X	(X) Optional	(X) Optional		Yes
3 and is suspected to meet Brown's criteria for severe [d]	Yes				X Consult with sponsor medical monitor	Yes
4 [d]	Yes or No				X Consult with sponsor medical monitor	Yes

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, version 4.03; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the subject to maintain the rAvPAL-PEG dose at the time of AE onset until improvement to grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The rAvPAL-PEG dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE grade ≥3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (grade 3) in the judgment of the investigator and the sponsor's medical monitor, the subject may be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE grade ≥3 hypersensitivity AE is related to administration with rAvPAL-PEG, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE [sampling must be performed >8 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, urinary N-methyl histamine, CRP, C3, C4, and tryptase).

9.1.1.3 Response to Anaphylaxis

If the investigator suspects that the event is anaphylaxis, the subject will be assessed in the clinic. Laboratory assessments for suspected anaphylaxis events should be performed prior to the next administration of study drug (if applicable) and include anti-rAvPAL IgE and

anti-rAvPAL-PEG IgE (sampling must be performed >8 hours after event onset and before the next dose of study drug) and tryptase (within 24 hours of event onset). The investigator will consult with the sponsor's medical monitor, and a decision will then be made whether to discontinue the subject from study drug, restart dosing at the same dose level, or restart dosing at a lower dose level.

For the first week of dosing after resolution of anaphylaxis, the study drug will be administered in a clinic setting with equipment for emergency resuscitation (including epinephrine) within easy access. The subject must be observed for 30 minutes following administration due to restarting dosing. Additionally, the subject should be premedicated with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to each dose of study drug for one week upon return to dosing regardless of the duration of dose interruption. If NSAIDs are administered as a premedication, they should be given with food.

9.1.2 Study Stopping Criteria

If an NCI-CTCAE grade ≥ 3 hypersensitivity treatment-related event occurs and meets Brown's criteria for severe (grade 3), an ad hoc independent DMC meeting will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct. Clinically severe hypersensitivity is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event:

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mm Hg (adults)
- Neurologic alteration: loss of consciousness, collapse, incontinence

Brown's severity criteria are presented in Table 9.1.2.1.

Table 9.1.2.1: Brown's Severity Criteria

Brown's Criteria for Hypersensitivity Reactions	Definition
Mild (1) skin and subcutaneous tissue	Generalized erythema, urticaria, periorbital edema, or angioedema
Moderate (2) features suggested respiratory, cardiovascular, or gastrointestinal involvement	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness (pre-syncope), diaphoresis, chest or throat tightness, or abdominal pain
Severe (3) hypoxia or neurologic compromise	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, loss of consciousness, or incontinence

9.1.3 Dose Modifications

9.1.3.1 Dose Increase Methodology

Each subject's dose may be modified based on the decision of the Investigator. Decisions regarding dose increases will depend on a subject's adverse event profile and blood Phe and drug concentrations.

An individual subject's dose may be adjusted beginning on Day 1 of PAL-003. For the duration of this study, an individual subject's dose may be adjusted if warranted per a subject's adverse event profile and blood Phe concentrations. Dose increases should be performed as follows:

- The dose may be increased by increments of no more than 10-fold higher than the subject's current dose.
- When increasing the dose, a dose may be used that is between the dose levels (0.001, 0.003, 0.01, 0.03, 0.06, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg or 2.5, 5, 10, 20, 40, 75, 150, 225, 300, 375 mg/week) or per Investigator determination based on available information regarding a subject's blood Phe (60-600 µmol/L) as well as any adverse events for an individual subject. Dose increases >350 mg/week require consultation with the sponsor's medical monitor.
- The dose may be adjusted by increasing the frequency up to daily-for a total weekly dose not to exceed 5.0 mg/kg or 375 mg/week. Dose increases > 350 mg/week require consultation with the sponsor's medical monitor.
- When a dose is increased, the subject must be observed for 30 minutes following the first modified dose administration. Subjects who increase their dose frequency do not need to be observed following injection of study drug if the increase in frequency does not increase the overall weekly dose amount.
- Only 1 dose adjustment is allowed every 2 weeks.

9.1.3.2 Dose Decrease Methodology

Decisions regarding dose decreases will depend on available information regarding a subject's blood Phe (60-600 µmol/L), as well as any adverse event (any hypersensitivity reaction as defined in Section 9.1.1). Dose decreases may occur for hypophenylalanemia or any adverse event that may be improved with a lower dose.

A dose should first be reduced by dose level (5.0, 4.0, 3.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, 0.06, 0.03, 0.01, 0.003, 0.001 mg/kg or 375, 300, 225, 150, 75, 40, 20, 10, 5, and 2.5 mg/week). It is also permitted to decrease dose in between these specified dose levels. If further dosing adjustments are required in response to safety, dosing frequency may also be adjusted.

If the subject develops low blood Phe (as defined by a blood Phe level ≤ 30 µmol/L), the subject's rAvPAL-PEG dose should be decreased to the next lower dose. If the condition persists after another 1 month at the lower dose, the dose should be decreased again to the next lower level. If the subject continues to experience low blood Phe after two dose level reductions, the subject may be asked to consult the dietician to eliminate medical foods and/or formulas and to ensure a balanced and nutritious diet (as part of the routine standard of care for PKU disease management, subject diet should be monitored by a dietician).

9.2 Discussion of Study Design, Including Choice of Control Group

This open-label, Phase 2 trial was designed to be closely monitored with the goal of obtaining information about the efficacy, safety, tolerability, and PK of long-term administration of rAvPAL-PEG in subjects with PKU. There will be no control group in this study.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

Individuals eligible to participate in this study must meet all of the following criteria:

1. Must have completed participation in a previous rAvPAL-PEG study.
2. Willing and able to provide written, signed informed consent, or in the case of subjects under the age of 18 years, provide written assent (if required) and written informed consent by a parent or legal guardian, after the nature of the study has been explained, and prior to any research-related procedures.
3. Willing and able to comply with all study procedures.

4. Females of childbearing potential must have a negative pregnancy test at Screening and be willing to have additional pregnancy tests during the study. Females considered not of childbearing potential include those who have been in menopause at least 2 years, or had tubal ligation at least 1 year prior to Screening, or who have had total hysterectomy.
5. Sexually active subjects must be willing to use two acceptable methods of contraception while participating in the study. Males post vasectomy for 2 years with no known pregnancies do not need to use any other forms of contraception during the study.
6. Maintained a stable diet.
7. In generally good health as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) at Screening.

9.3.2 Exclusion Criteria

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Use of any investigational product (with the exception of rAvPAL-PEG) or investigational medical device within 30 days prior to Screening, or requirement for any investigational agent prior to completion of all scheduled study assessments.
2. Use of any medication other than rAvPAL-PEG that is intended to treat PKU within 14 days prior to the administration of study drug.
3. Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, within 6 months prior to Screening and during study participation.
4. A prior reaction that included systemic symptoms (eg, generalized hives, respiratory or gastrointestinal problems, hypotension, angioedema, anaphylaxis) to rAvPAL-PEG or a PEG-containing product. Subjects with a prior systemic reaction may be eligible for participation per the discretion of the Principal Investigator in consultation with the sponsor's medical monitor. However, subjects who discontinued from study drug early due to a reaction are not eligible to enroll into this study.
5. Pregnant or breastfeeding at Screening or planning to become pregnant (self or partner) or to breastfeed at any time during the study.
6. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

7. Any condition that, in the view of the PI, places the subject at high risk of poor treatment compliance or of not completing the study.
8. Known hypersensitivity to rAvPAL-PEG necessitating early termination from a previous rAvPAL-PEG study or known hypersensitivity to any of its excipients.
9. Alanine aminotransferase (ALT) concentration > 2 times the upper limit of normal.
10. Creatinine > 1.5 times the upper limit of normal.

9.3.3 Removal of Subjects from Treatment or Assessment

Subjects (or their legally authorized representative) may withdraw their consent to participate in the study or to receive study drug at any time without prejudice. The Investigator must withdraw from the study or study drug treatment any subject who requests to be withdrawn. A subject's participation in the study may be discontinued at any time at the discretion of the Investigator and in accordance with his/her clinical judgment. When possible, a subject who is discontinued from study drug should continue to perform the study tests and evaluations until study completion. For subjects who discontinue from the study, the tests and evaluations listed for the Early Termination Visit should be carried out (refer to Section 12.4).

BioMarin must be notified of all subject withdrawals as soon as possible. BioMarin also reserves the right to discontinue the study at any time for either clinical or administrative reasons and to discontinue participation by an individual Investigator or site for poor enrollment or noncompliance.

Reasons for which the Investigator or BioMarin may withdraw a subject from the study drug include, but are not limited to, the following:

- Subject experiences a serious or intolerable adverse event (AE).
- Subject develops a clinically significant laboratory abnormality.
- Subject requires medication prohibited by the protocol.
- Subject becomes pregnant (refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy and refer to Section 9.1 for details on re-introduction of study drug following pregnancy and breastfeeding).

Reasons for which the Investigator or BioMarin may withdraw a subject from the study include, but are not limited to, the following:

- Subject does not adhere to study requirements specified in the protocol.
- Subject was erroneously admitted into the study or does not meet entry criteria.
- Subject is lost to follow-up.

If a subject fails to return for scheduled visits, a documented effort must be made to determine the reason. If the subject cannot be reached by telephone within 7 days, a certified letter should be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

The Investigator or designee must explain to each subject, before enrollment into the study, that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and IRB. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, from each subject, or if appropriate, the subject's legally authorized representative. If permission to use protected health information is withdrawn, it is the Investigator's responsibility to obtain a written request, to ensure that no further data will be collected from the subject and the subject will be removed from the study.

9.3.4 Subject Identification and Replacement of Subjects

Subjects will retain the same subject number assigned in their previous rAvPAL-PEG study. This unique identifier will be on all case report form (CRF) pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used.

Subjects who do not complete the study will not be replaced.

9.4 Treatments

Upon enrollment into this study, subjects from previous rAvPAL-PEG studies will be dosed with the same or higher dose that was administered upon completion of that study provided that there was no interruption in dosing. rAvPAL-PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be adjusted per Investigator determination based on available information regarding a subject's blood Phe level (60-600 µmol/L), as well as any adverse events (any hypersensitivity reaction) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 weeks will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

Subjects may self-administer study drug at home if approved by the Investigator and the sponsor's medical monitor and if adequate training is demonstrated. Subjects may be eligible to self-administer study drug if he or she meets the following criteria:

- The subject has no cognitive impairments that may increase the safety risk of self-administration per the assessment of the investigator.
- The subject has been approved for self-administration of study drug by the sponsor's medical monitor.
- The subject has completed the Self-Administration Training conducted by qualified study site personnel and has been observed by the study site personnel to be able to adequately prepare the proper dose and perform the injections safely.
- The subject has been provided with two epinephrine injectors and has been trained on when and how to administer it. The subject will be instructed to carry one epinephrine injector with them at all times.

Qualified study site personnel will train each eligible subject on all procedures for self-administration of study drug (vial and syringe and prefilled syringe drug products) under the supervision of the PI. Qualified study site personnel will also be required to observe the subject prepare and perform at least 1 injection prior to allowing the subject to self-administer a dose at home. The subject will see a study site nurse or home healthcare nurse in person every week or receive a telephone call from site staff to ensure that the subject continues to perform all self-administration procedures correctly, to assess adverse events, and to answer questions throughout the duration of the study. The study site nurse or home healthcare nurse will also perform the regularly scheduled assessments and procedures as outlined in Table 9.1.1 and Section 12; the subject must perform a clinic visit monthly at minimum. Training materials and other information specific to the study site personnel are provided in the Subject Self-Administration Training Materials. Prefilled syringes are dispensed to subjects only after the subject (or caregiver) has completed training and competency is demonstrated in the clinic. Additional regular refresher training occurs in the clinic, every 24 weeks, and more often as needed.

Subjects who are eligible for self-administration will be provided with a Subject Study Manual. Self-administration training materials will be provided in the Subject Study Manual to supplement the in-person training provided by the qualified study site personnel. The Subject Study Manual will include step-by-step instruction on the following:

- How to prepare and perform the injections of study drug safely.
- How to receive, track, store, prepare, and return both used and unused study drug.
- How to safely use and dispose of syringes, including prefilled syringes, used for injections of study drug.
- How to use a new syringe and vial or new prefilled syringe every time drug is administered.

- How to care for their injection site after an injection of study drug.
- How to identify an adverse reaction and how to report this reaction to the study site.
- How and when to use epinephrine injectors.
- Who to contact at the study site in case of an emergency.

The PI or the sponsor's medical monitor may request that self-administration be halted at any time and that the subject resume injections performed by the study site personnel or home healthcare nurse.

Subjects who are within the target Phe range may also be permitted to convert from weight-based dosing to fixed dosing (refer to Section 9.4.4).

9.4.1 Treatments Administered

BioMarin and/or its designee will provide the study site or subject with a supply of IP sufficient for the completion of the study. BioMarin is responsible for shipping study drug to the clinical sites. The clinical site is responsible for supplying the study drug to subjects who qualify for self-administration. Refer to the PAL-003 Pharmacy Manual for information pertaining to the distribution of study drug from sites.

Information regarding supplying study drug to subjects who qualify for self-administration is provided in the Subject Self-Administration Training Materials.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

The investigational product is rAvPAL-PEG (recombinant Anabaena variabilis phenylalanine ammonia lyase-PEG). rAvPAL-PEG will be provided in vials or prefilled syringes; subjects will be supplied with vials and syringes or prefilled syringes for administration in the clinic and self-administration:

- rAvPAL-PEG will be provided in 3 mL (milliliter) vials, each containing 1.5 ± 0.2 mL to deliver 15 mg of rAvPAL-PEG per 1.0 mL (15 mg/mL protein concentration). Each vial is filled with 1.5 ± 0.2 mL to allow the withdrawal and delivery of up to 1.0 mL of the drug product.

Or

- rAvPAL-PEG will be provided in 3-mL vials, each containing 1.8 ± 0.3 mL to deliver 20 mg of rAvPAL-PEG per 1.3 mL (15 mg/mL protein concentration). Each vial is filled with 1.8 ± 0.3 mL to allow withdrawal and delivery of up to 1.3 mL.

Or

- rAvPAL-PEG will be provided in prefilled syringes in three sizes that deliver up to 1 mL. Syringe sizes are 2.5 mg (5 mg/mL protein concentration), 10 mg (20 mg/mL protein concentration), and 20 mg (20 mg/mL protein concentration).

Table 9.4.2.1.1: Prefilled Syringe Volume, Concentration, and Dose

Prefilled Syringe	Volume	Concentration	Dose
Sku #1	0.5 mL	5 mg/mL	2.5 mg
Sku #2	0.5 mL	20 mg/mL	10 mg
Sku #3	1.0 mL	20 mg/mL	20 mg

Subjects who have undergone training and who have demonstrated competency on the use of prefilled syringe in the study clinic may be eligible to transition from vial and syringe to prefilled syringe dosing. Additionally, regular refresher training on use of prefilled syringe will be provided in study clinic every 24 weeks, and more often as needed.

Subjects should convert their vial and syringe dose to prefilled syringe dose using the following table:

Table 9.4.2.1.2: Conversion from Vial and Syringe Dose to Prefilled Syringe Dose

Daily Dose (mg) Range	Daily Dose (mg) Round To[a]	# of 2.5mg Prefilled Syringe Needed	# of 10mg Prefilled Syringe Needed	# of 20mg Prefilled Syringe Needed
0 – 3.7	2.5	1	0	0
3.8 – 6.2	5	2	--	--
6.3 – 8.7	7.5	3	--	--
8.8 – 11.2	10	--	1	--
11.3 – 13.7	12.5	1	1	--
13.8 – 16.2	15	2	1	--
16.3 – 18.7	17.5	3	1	--
18.8 – 21.2	20	--	--	1
21.3 – 23.7	22.5	1	--	1
23.8 – 26.2	25	2	--	1
26.3 – 28.7	27.5	3	--	1
28.8 – 31.2	30	--	1	1
31.3 – 33.7	32.5	1	1	1
33.8 – 36.2	35	2	1	1
36.3 – 38.7	37.5	3	1	1
38.8 – 41.2	40	--	--	2
41.3 – 44.9	42.5	1	--	2
45 – 54.9	50	--	1	2
55 – 64.9	60	--	--	3
65 – 74.9	70	--	1	3
75 – 84.9	80	--	--	4
85 – 94.9	90	--	1	4
95 – 104.9	100	--	--	5
105 – 114.9	110	--	1	5
115 – 124.9	120	--	--	6
125 – 134.9	130	--	1	6
135 – 144.9	140	--	--	7
145 – 154.9	150	--	1	7

[a] For <45 mg/day, round to nearest 2.5 mg; for ≥45 mg/day, round to nearest 10 mg.

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, L-phenylalanine, and water for injection.

rAvPAL PEG doses will be administered SC by either health care professionals or via self-administration. The dose level may be increased or decreased per Investigator determination based on available information regarding a subject's blood Phe level (60 600 µmol/L), as well as any adverse events (any hypersensitivity AE) for an individual subject. The dosage that provides control of blood Phe concentrations within the target range of 60-600 µmol/L for a minimum of 2 consecutive measurements will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses.

For vial and syringe, drug product packaging will be identified with the lot number and expiration date and will be provided in a box labeled with the study number.

For prefilled syringe, drug product packaging will be identified with the size (2.5 mg, 10 mg, or 20 mg), lot number, and kit identification (ID). The expiration date will be available in a separate Certificate of Compliance issued for each lot.

9.4.3 Storage

IP must be stored at 5 ± 3°C (41 ± 5°F) under the conditions specified in the IB. At the site, the IP will be stored in a secure area accessible only to the designated pharmacists and clinical site personnel. All IP must be stored and inventoried and the inventories must be carefully and accurately documented according to applicable state, federal and local regulations, ICH GCP, and study procedures.

Specific instructions for storage of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.4 Directions for Administration

Doses will be administered SC and the favorable dose will be determined for each subject by adjusting the dose level, dose frequency, or both, per the PI, based upon the subject's response to doses. Dosing is up to a maximum dose per week of 5.0 mg/kg (or 375 mg for subjects receiving a fixed dose).

For subjects receiving weight-based dosing, dosage is calculated by multiplying each subject's weight in kilograms (kg) on Day 1 (predose) by the assigned dose (in mg/kg). During the study, if the weight of a subject changes more than 10% from the measurement obtained at baseline or at a previous measurement, the subject's dose may be adjusted to

accommodate the change. The change must be recorded. Refer to the Pharmacy Manual for additional information on calculating dosage.

Subjects who have demonstrated efficacy (as measured by blood Phe within the target range of 60-600 µmol/L for at least 2 consecutive measurements) and who have maintained a stable rAvPAL-PEG dose for at least 2 consecutive measurements may be eligible to transition from weight-based dosing to a fixed dosing regimen that would permit daily dosing (5- or 7-days-per-week). Subjects who convert to a fixed-dosing regimen should convert their dose based upon the information presented in Table 9.4.4.1.

Table 9.4.4.1: Fixed Doses for Subjects on Weight-Based Dose Regimen

Weight-based dose (mg/kg)	Equivalent fixed dose (mg)	Vol for 15 mg/ml concentration (ml) (vial)[a]
0.03	2.5	0.17
0.06	5	0.33
0.12	10	0.67
0.25	20	1.33
0.5	40	2.67
1.0	75	5
2.0	150	10
3.0	225	15
4.0	300	20
5.0	375	25

[a] Applies to both 1.0 and 1.3 ml withdrawal.

It is preferable that the injection sites should be alternated between doses, ie, upper left arm in Week 1, upper right arm in Week 2, etc. Doses may be administered in any of the common SC areas (upper arm, thigh, abdomen). If dose volume is such that a dose will be divided and given in more than 1 injection site, it is preferable to use both arms, both legs, or both sides of the abdomen.

Subjects may require more than 1 injection per dose, depending upon site policy (ie, some institutions do not permit a single SC injection of greater than 2 mL). Dosage calculations must be performed according to study drug preparation instructions provided in the PAL-003 Pharmacy Manual. Study drug will be administered by clinic staff, other qualified and trained study personnel, or qualified subjects.

Information for dosing of subjects who enroll into this study after completing previous rAvPAL studies is provided in Section 9.1. Instructions for subjects who re-start rAvPAL-PEG dosing due to unplanned missed doses are provided in Section 9.4.10.

Instructions for administration of study drug for subjects who qualify for self-administration are provided in the Subject Self-Administration Training Materials.

9.4.5 Method of Assigning Subjects to Treatment Groups

This will be an open-label study; no randomization schedule will be generated.

Subjects will retain the same subject number used in their previous rAvPAL-PEG study.

9.4.6 Selection of Doses Used in the Study

This Phase 2 study aims to evaluate the efficacy, safety, tolerability, and PK parameters of multiple doses of rAvPAL-PEG after long-term administration in subjects with PKU. A dose that produces a reduction in blood Phe concentrations to 60-600 µmol/L will be determined for each subject based on preliminary safety, antibody, and efficacy data from this ongoing study.

9.4.6.1 Selection of Timing of Dose for Each Subject

Study drug will be administered by clinic staff or other qualified and trained study personnel.

Subjects may self-administer study drug at home if approved by the sponsor's medical monitor and if adequate training is provided (refer to Section 9.4).

9.4.7 Blinding

This is an open-label study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter (OTC) medications taken by a subject for 30 days before Screening will be recorded on the designated CRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol. In the event of an emergency, any needed medications may be prescribed without prior approval, but the sponsor's medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the CRF.

Use of any investigational product (with the exception of rAvPAL-PEG used in a previous rAvPAL-PEG study) or investigational medical device within 30 days before Screening, or

requirement for any investigational agent prior to completion of all scheduled study assessments, is prohibited.

Use or planned use of any injectable drugs containing PEG (other than rAvPAL-PEG), including Depo-Provera, is prohibited within 6 months prior to Screening and during study participation. A list of PEG-containing injectable drugs is provided in the study reference materials. Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product will be encouraged to be premedicate as outlined in Section 9.1.1. If the hypersensitivity AE worsens with a repeat injection (as determined by the Investigator in consultation with the sponsor's medical monitor) even with premedication prior to study drug dosing, the subject must be withdrawn from the study and complete an Early Termination Visit.

Subjects who have had a prior hypersensitivity AE to rAvPAL-PEG or a PEG-containing product may participate in this study (refer to Section 9.3.2).

Use of any medication that is intended to treat PKU within 14 days prior to the administration of study drug is prohibited.

9.4.9 Treatment Compliance

The date, time, volume, and concentration of each dose of study drug administered to each subject must be recorded in the dispensing log provided for the study, as well as on the appropriate CRF. This data will be used to assess treatment compliance.

9.4.10 Dose Interruption and Missed Doses

Information for dosing of subjects who enroll into this study after completing previous rAvPAL-PEG studies is provided in Section 9.1.

During this long-term extension study, interruption of rAvPAL-PEG dosing may occur per subject decision or per the Investigator. After a dosing interruption of ≥ four consecutive doses, rAvPAL-PEG administration should be re-started at the same dose level and dosing frequency that was administered prior to the dosing interruption. The first week of dosing of rAvPAL-PEG following a dose interruption should be administered in a clinic setting, and the subject must be observed for 30 minutes following administration due to restarting dosing. Premedication with H1 antagonist, H2 antagonist, and antipyretics (eg, acetaminophen) approximately 2-3 hours prior to study drug should be administered following any dose interruption of ≥ four consecutive doses. If NSAIDs are administered as a premedication, they should be given with food. A follow-up telephone call will be made to the subject to monitor subject safety 24 hours following the return to dosing. Subjects should

continue to perform the study assessments as outlined in the Schedule of Events (refer to Table 9.1.1) during any dosing interruption.

Subjects who miss rAvPAL-PEG dosing due to poor compliance may be discontinued from further study drug per discretion of the Sponsor or Investigator (refer to Section 9.3.3).

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject-by-subject dose specific accounting), IP returned, and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor (on-site CRA) has confirmed the accountability data.

9.5.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after BioMarin has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to BioMarin and retained in the PIs study files. If a site is unable to destroy study drug appropriately, the site can return unused study drug to BioMarin upon request. The return of study drug or study drug materials must be accounted for on a Study Drug Return Form provided by BioMarin.

Subjects who qualify for self-administration of study drug will be provided with instructions for returning used and unused study drug and the proper disposal of any study drug materials (refer to the Subject Self-Administration Training Materials).

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.6 Dietary or Other Protocol Restrictions

Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician.

A 3-day dietary record will be completed by subjects and will be brought to clinic visits for review with clinic staff. It is preferable that subjects complete the 3-day record immediately prior to their next monthly study visit. The dietary record will be maintained with the study source documents. Information regarding a subject diet diary is provided in Section 9.7.4.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedules of Events in the Synopsis describe the timing of required evaluations.

For the laboratory assessments, the party responsible for performing the assessment and the pertinent protocol section describing the details of the test are listed in Table 9.7.1.1.

Table 9.7.1.1: Summary of Laboratory Assessments

Laboratory Assessment	Party Responsible for Performing Test	Section in Protocol for Details
Clinical laboratory tests	Central laboratory	9.7.6
Antibody testing	BioMarin[a] or CRO	9.7.5.2
PK variables	BioMarin	9.7.3
Blood Phe concentrations	Central laboratory	9.7.2.1
Pregnancy test (urine) and sedimentation rate	Local laboratory	9.7.6, 9.7.5.1
Urinalysis	Central laboratory	9.7.6

Phe, phenylalanine; PK, pharmacokinetic.

[a]Analysis of the anti-rAvPAL IgE and anti-rAvPAL-PEG IgE assay will be performed by a Contract Research Organization.

9.7.1.1 Demographic Data and Medical History

Demographic data and a detailed medical history, including allergy history, will be obtained at Screening. The medical history will include specific information concerning any prior or existing medical conditions that might interfere with study participation or safety.
This medical history should elicit all major illnesses, diagnoses, and surgeries that the subject has ever had, and whether the subject has a history of alcohol and/or drug abuse.

9.7.1.2 Physical Examination Findings

Physical examination will include assessment of general appearance; CV; dermatologic; head, eyes, ears, nose, and throat; lymphatic; respiratory; GI; musculoskeletal; and neurological/psychological. Other body systems may be examined. Day 1 results will be the baseline values and clinically significant changes from baseline will be recorded as an AE.

9.7.1.3 Vital Sign Measurements

Vital signs will be measured after resting for 5 minutes, and include seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C). Height (cm) will be measured at Screening only. Weight will be measured at Screening and then monthly.

9.7.1.4 Electrocardiography

A standard 12-lead ECG will be recorded while the subject is resting, at Screening and at the Final Follow-Up (F/U) Visit or Early Termination Visit (ETV). If clinically significant abnormalities are noted at Screening, the PI or designee is required to assess whether it is appropriate for the subject to enroll in the study.

9.7.1.5 Chest X-Ray

A chest X-ray will be performed at the Screening, Week 48, and Final Follow-Up (or Early Termination) visits to assess gross pulmonary health.

9.7.2 Efficacy Variable

9.7.2.1 Blood Phenylalanine Concentrations

Blood samples for Phe concentration measurements will be drawn monthly, at least 2.5 hours after a meal, on the days and time points indicated in the Schedule of Events (Table 9.1.1).

Blood Phe may be collected more often than monthly at the discretion of the Investigator should more frequent monitoring of blood Phe levels be clinically required.

A central laboratory will be used for blood Phe concentration analysis.

9.7.3 Pharmacokinetic Variables

Subjects enrolled in the Substudy will have additional PK sampling performed. For subjects participating in the Substudy, PK sampling will be performed as follows:

- For subjects who are administered rAvPAL-PEG once per week, plasma PK sampling will be performed pre-dose and every 24 hours thereafter (24, 48, 72, and 96 hours if possible), and then pre-dose of the next weekly dose.
- For subjects who are administered rAvPAL-PEG two or three times per week, plasma PK sampling will be performed pre-dose and every 24 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

- For subjects who are administered rAvPAL-PEG four to seven times per week, plasma PK sampling will be performed pre-dose and every 12 hours during the longest period between doses, when possible, and then pre-dose of the next dose.

See Table 9.1.2 for additional details.

BioMarin will perform the analysis.

9.7.4 Exploratory Efficacy Variable

Diet will be assessed for its role in blood Phe reduction relative to rAvPAL-PEG dosing using a 3-day diet diary, which will be given to subjects to complete at home. Subjects will bring the completed diary with them to clinic visits on a monthly basis. Subjects will be instructed to record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days each month. If changes in diet are noted per the information on the diet diary, the subject will be instructed to maintain their diet as reported at the beginning of the study or as otherwise instructed by the Investigator.

9.7.5 Safety Variables

9.7.5.1 Pregnancy Testing

Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests. Female subjects with a positive serum pregnancy test at Screening do not meet eligibility criteria for enrollment.

Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. Serum pregnancy tests will be performed in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 10.3 for details on the reporting procedures to follow in the event of pregnancy.

9.7.5.2 Antibody Testing

Immunogenicity will be assessed by determining immune response and neutralizing activity with the following measurements: anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE, and anti-rAvPAL-PEG neutralizing antibodies (refer to Section 9.1.1). Immunogenicity assays will be performed at the time points indicated in the Schedule of Events (Table 9.1.1).

BioMarin will perform all antibody testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.

9.7.6 Clinical Laboratory Assessments

Any abnormal test results determined to be clinically significant by the Investigator should be repeated (at the Investigator's discretion) until the cause of the abnormality is determined, the value returns to baseline or to within normal limits, or the Investigator determines that the abnormal value is no longer clinically significant. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at baseline. Subjects who had elevated results at baseline followed by a confirmed subsequent increase of 100-200 mg/g from baseline should also be referred to a nephrologist for consultation.

All abnormal clinical laboratory result pages should be initialed and dated by an Investigator, along with a comment regarding whether or not the abnormal result is clinically significant.

The diagnosis, if known, associated with abnormalities in clinical laboratory tests that are considered clinically significant by the Investigator will be recorded on the AE CRF.

Table 9.7.6.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests[a]	Other
Albumin	Hemoglobin	Appearance	Pregnancy test, if applicable[a]
Alkaline Phosphatase	Hematocrit	Color	
ALT (SGPT)	WBC count	pH	Phenylalanine
AST (SGOT)	RBC count	Specific gravity	Tyrosine
Total bilirubin	Platelet count	Ketones	Sedimentation rate[a]
BUN	Differential cell count	Protein	
Creatinine		Glucose	
GGT		Spot urine albumin/creatinine ratio [b]	**Additional Unscheduled Hypersensitivity Reaction Visit Tests[b]**
Total protein		Nitrite	CH50
Calcium		Urobilinogen	C_1, C_3, C_4
Sodium		Hemoglobin	Serum tryptase level[b]
Potassium		Bilirubin	CRP
Glucose			Urine N-methyl histamine
			Urine albumin/creatinine ratio [c]
Uric acid			**Complement Testing[d]**
CO_2			C_3
Chloride			C_4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, complement; CH50, total hemolytic complement; CO2, carbon dioxide; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory. It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[b] Perform within 24 hours of event onset.

[c] It is recommended that urine samples are obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at baseline. Subjects who had elevated results at baseline followed by a confirmed subsequent increase of 100-200 mg/g from baseline should also be referred to a nephrologist for consultation.

[d] Complement C_3 and C_4 to be drawn at the Screening Visit and then every 8 weeks or as needed to resolve abnormal test results.

9.7.7 Exploratory Sample Analyses

Blood and urine samples will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. For each portion of the blood and urine samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study, unused sample portions may be used during the study or after the subject's study completion for assay development or other purposes.

All samples collected in this study may be used for exploratory research. In addition, samples collected for other purposes may be used for exploratory research use once the primary use has been completed.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

For this protocol, a reportable adverse event (AE) is any untoward medical occurrence (e.g., sign, symptom, illness, disease or injury) in a subject administered the study-drug or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the course of the study.
- Pre-existing medical conditions judged by the Investigator to have worsened in severity or frequency or changed in character during the study.
- Complications that occur as a result of non-drug protocol-imposed interventions (eg, AEs related to screening procedures, medication washout, or no-treatment run-in).

An adverse drug reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. “Reasonable possibility” means there is evidence to suggest a causal relationship between the study-drug and the AE.

Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The study AE reporting period is as follows: After informed consent but prior to initiation of study drug, only SAEs associated with any protocol-imposed interventions will be reported. After informed consent is obtained and the first administration of study drug, all non-serious AEs and SAEs reporting period begins and continues until 4 weeks following the last administration of study drug or the Study Completion Visit/Early Termination Visit, whichever occurs last (refer to Section 12). The criteria for determining, and the reporting of SAEs is provided in Section 10.2.

For this study, a medical device is defined as the prefilled syringe and all of the component parts. The reporting period for device-related events begins with the first dose administered using prefilled syringe and ends with the last dose administered using prefilled syringe. Events assessed by the investigator as related to the device, including malfunction, injury, or medication error, will be captured into an electronic data capture system. All serious events related to the device will be reported to BioMarin Pharmacovigilance (BPV) within 24 hours using the device-related event report form and entered onto the appropriate eCRF page(s) as required.

The Investigator should follow all unresolved AEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study drug or study participation is not the cause of the AE. Outcome of AEs (and resolution dates) should be documented on the appropriate CRF page(s) and in the subject's medical record unless the subject is lost to follow-up or it has been determined that the study drug or study participation is not the cause of the AE.

The Investigator responsible for the care of the subject or qualified designee will assess AEs for severity, relationship to study-drug, and seriousness (see Section 10.2 for SAE definition). Severity (as in mild, moderate or severe headache) is not equivalent to seriousness, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

The Investigator will determine the severity of each AE using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4 as stated below.

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (ADL)[b]
4	Life threatening or debilitating: consequences; urgent intervention indicated
5	Death related to AE

Grade 4 and 5 AEs should always be reported as SAEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

The Investigator will determine the relationship of an AE to the study drug and will record it on the source documents and AE CRF, using the categories defined below.

Relationship	Description
Not Related	Exposure to the IP has not occurred OR The administration of the IP and the occurrence of the AE are not reasonably related in time OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Possibly Related	The administration of the IP and the occurrence of the AE are reasonably related in time AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Probably Related	The administration of IP and the occurrence of the AE are reasonably related in time AND The AE is more likely explained by exposure to the IP than by other factors or causes.

In order to classify AEs and diseases, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using MedDRA (Medical Dictionary for Regulatory Activities terminology).

Factors suggestive of a causal relationship could include (but are not limited to) the following:

- Plausible temporal relationship
- Absence of alternative explanations
- Rarity of event in a given patient or disease state
- Absence of event prior to study drug exposure
- Consistency with study product pharmacology
- Known relationship to underlying mechanism of study drug action
- Similarity to adverse reactions seen with related drug products
- Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose meets 1 or more of the following criteria:

- Is fatal.
- Is life threatening.
    - Note: Life-threatening refers to an event that places the subject at immediate risk of death. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires or prolongs inpatient hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect in the child or fetus of a subject exposed to IP prior to conception or during pregnancy.
- Important medical events or reactions that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes above should also usually be considered serious.

For this protocol, anaphylaxis per NIAID/FAAN criteria for the clinical diagnosis of anaphylaxis is designated as an AE of special interest (serious or nonserious and irrespective of severity) to facilitate rapid reporting and sponsor review. All occurrences of anaphylaxis per NIAID/FAAN criteria will be reported to the sponsor within 24 hours of the site becoming aware of the event using the SAE form. Severity and serious criteria (if applicable) should be reported on the SAE form.

The reporting period for SAEs related to protocol imposed interventions begins after informed consent is obtained, the reporting period for all SAEs begins after first dose of study drug and continues until 4 weeks following the last administration of study drug or End of Treatment Visit. The reporting period for Serious Device Related Events begins after first dose of study drug and ends with the last administered dose.

All SAEs (including serious device-related events), whether or not considered related to study drug, must be reported within 24 hours of the site becoming aware of the event by faxing the study-specific SAE Report Form or device Report Form to BioMarin Pharmacovigilance (BPV). Each SAE must also be reported in the CRF Investigators should not wait to collect information that fully documents the event before notifying BPV of a SAE. BioMarin may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators

submit any information requested by BioMarin as soon as it becomes available. The Investigator should follow all unresolved SAEs until the events are resolved or stabilized, the subject is lost to follow-up, or it has been determined that the study drug or participation is not the cause of the AE. Resolution of AEs (with dates) should be documented in the CRF and in the subject's medical record.

For some SAEs, the Sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Reporting of SAEs to the IRB will be done in compliance with the standard operating procedures and policies of the IRB and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB was properly and promptly notified as required.

10.3 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the eCRF. For example, if severe hemorrhage leads to renal failure, both events should be recorded separately on the eCRF.

10.4 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between subject evaluation time points. Such an event should be recorded only once on the eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF).

A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be individually recorded as a separate event on the AE eCRF.

10.5 Abnormal Laboratory Values

Laboratory test results will be recorded on the laboratory results pages of the eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable.

Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the eCRF.

A clinical laboratory abnormality should be documented as AE if it is not otherwise refuted by a repeat test to confirm the abnormality and any one or more of the following conditions is met:

- Accompanied by clinical symptoms
- Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
- Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment).
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria.

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.

10.6 Pre-existing Conditions

A pre-existing condition is one that is present at the start of the study. Such conditions should be recorded as medical history on the appropriate eCRF.

A pre-existing condition should be recorded as an AE or SAE during the study only if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, more frequent headaches).

10.7 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.6). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.

10.8 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2).

There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to the following:

- Perform a protocol-mandated efficacy measurement
- Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed in severity
- Receive scheduled therapy (study drug or otherwise) for the study indication

10.9 Deaths

All deaths that occur during the AE reporting period (refer to Section 10.1 for nonserious AEs and Section 10.2 for SAEs), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” or “Death of Unknown Cause” on the eCRF.

10.10 Pregnancy

Pregnancy in a subject or partner should be reported within 24 hours of the site becoming aware of the pregnancy to BioMarin Pharmacovigilance by fax using the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose. In addition, pregnancy in a subject is also reported on the Subject Disposition eCRF and the End of Study eCRF if the subject withdraws from the study due to pregnancy. The Investigator must make every effort to follow the subject through resolution of the pregnancy (delivery or termination) and to report the resolution to BPV in the study reference materials. In the event of pregnancy in the partner of a study subject, the Investigator should make every reasonable attempt to obtain the woman’s consent for release of protected health information.

Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the Sponsor as an SAE.

10.11 Follow-up of Subjects after Adverse Events

For some SAEs and AESIs, the sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.

10.12 Post-Study Adverse Events

At the last scheduled visit, the investigator should instruct each subject to report to the investigator and/or to BPV directly any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment.

The investigator should notify the study sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if the investigator believes that the death or SAE may have been related to prior study treatment. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject who participated in this study.

10.13 Urgent Safety Measures

The regulations governing clinical trials state that the sponsor and Investigator are required to take appropriate urgent safety measures to protect subjects against any immediate hazards that may affect the safety of subjects, and that the appropriate regulatory bodies should be notified according to their respective regulations. According to the European Union (EU) Clinical Trial Directive 2001/20/EC, "…in the light of the circumstances, notably the occurrence of any new event relating to the conduct of the trial or the development of the investigational medicinal product where that new event is likely to affect the safety of the subjects, the sponsor and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. The sponsor shall forthwith inform the competent authorities of those new events and the measures taken and shall ensure that the IRB/EC/REB is notified at the same time." The reporting period for these events which may require the implementation of urgent safety measures is the period from the time of signing of the ICF through the completion of the last study visit or at the ETV. Investigators are required to report any events which may require the implementation of urgent safety measures to BioMarin with 24 hours.

Examples of situations that may require urgent safety measures include discovery of the following:

- Immediate need to revise the IP administration (i.e., modified dose amount or frequency not defined in protocol).
- Lack of study scientific value, or detrimental study conduct or management.
- Discovery that the quality or safety of the IP does not meet established safety requirements.

10.14 BioMarin Pharmacovigilance Contact Information

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: (415) 506-6179
Fax: (415) 532-3144
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the Sponsor's Medical Office sponsor's medical monitor r any AEs for which the issue of seriousness is unclear or questioned. Contact information for the sponsor's medical monitor is as follows:

Name: [redacted] MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

11 APPROPRIATENESS OF MEASUREMENTS

11.1 Safety and Pharmacokinetics

The PK and safety assessments in this study are used widely and are generally recognized as reliable, accurate, and relevant.

11.2 Diet Diary

A 3-day diet diary will be issued to subjects for completion and will be brought to clinic on a monthly basis for review with the clinical study staff. Additional information regarding the diet diary is provided in Section 7.3, and information regarding administration of the diary is provided in Section 9.7.4. Additional information about diet is provided in Section 9.1 and Section 9.6.

12 STUDY PROCEDURES

12.1 Prestudy

An ICF must be signed and dated by the subject (or the subject's legally authorized representative if the subject is less than 18 years of age), the PI or designee and witness (if required) before any study-related procedures are performed.

12.2 Screening Visit

After subjects have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening:

- Medical history, including allergy history, and demographics
- Physical examination
- Vital signs, including height and weight
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Chest X-ray
- 12-lead ECG
- Clinical laboratory tests (including spot urine albumin/creatinine ratio, hematology, chemistry, and urinalysis)
- Complement C_3 and C_4
- Assessment of SAEs
- Concomitant medications
- Diet query
- Blood Phe and plasma tyrosine concentration

For subjects who participated in a previous rAvPAL-PEG study, these assessments may be the same as those used for the last visit of the previous study, if they occur within 28 days from Day 1.

12.3 Treatment Period

During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications

assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. If there is a skin reaction that lasts ≥14 days, the Skin Reaction eCRF should be completed and the subject should be referred to a dermatologist for consultation and a skin biopsy. It is recommended that a photograph of the skin reaction may be taken by the subject or the site to help assess the event; photographs may be collected by the sponsor.

If the dose is modified by increasing dose level, additional blood Phe concentration testing should be done daily for 3 days following dose adjustment. If dose is modified by increasing either dose frequency or dose level, additional blood Phe concentration testing should be done prior to each dose for the first 2 weeks following dose adjustment.

12.3.1 Day 1 (Week 1)

Within 28 days of completing screening assessments, the following study activities will be performed on Day 1:

- Physical examination
- Vital signs
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- Administer study drug (study drug may be administered on additional days during the week as determined by the Investigator)

12.3.2 Weekly Telephone Visit

Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled visits every 8 weeks per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:

- Injection-site self inspection (previous and current injection site)

- Assessment of AEs
- Concomitant medications
- Confirmation of dose level, frequency, and study drug administration

12.3.3 Substudy Visits

For subjects participating in the PK substudy, refer to Section 9.7.3 and Table 9.1.2.

12.3.4 Every 8 Weeks

Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit.

Visits must be performed in the clinic every 8 weeks. The following study activities will be performed:

- Physical examination
- Vital signs
- Sedimentation rate
- Weight
- Clinical laboratory tests
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) For safety reasons, this test must be performed prior to the chest X-ray.
- Chest x-ray (Week 48 visit only)
- Complement C_3 and C_4
- Assessment of AEs
- Concomitant medications
- Diet query
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies)
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the next monthly visit.)

- Prefilled syringe self-administration training (to be performed prior to subjects transitioning from vial and syringe drug product to prefilled syringe drug product).
- Administer study drug
- Prefilled syringe refresher training (every 24 weeks only)
- If additional training is needed on days subsequent to the 8-week scheduled visit, additional clinic visit(s) or HHRN may be used.

12.3.5 Unscheduled Hypersensitivity Reaction Visit

Subjects who have an NCI-CTCAE grade ≥ 3 hypersensitivity reaction after rAvPAL-PEG administration (refer to Section 9.1.1) will have assessments performed immediately following (within 24 hours) the reaction.

- Vital signs
- Clinical laboratory tests
- Urine/albumin creatinine ratio
- CRP, CH50, C1, C3, and C4
- Urine N-methyl histamine
- Serum anti-rAvPAL IgE and anti-rAvPAL-PEG IgE only (performed > 8 hours after event onset)
- Serum tryptase level
- Skin biopsy (optional; affected and not affected area; to be performed within 1 week of the reaction)
- Assessment of AEs
- Concomitant medications

Following completion of the Unscheduled Hypersensitivity Reaction visit, subjects may resume study drug administration per the discretion of the Principal Investigator and the sponsor's medical monitor.

12.4 Study Completion/Early Termination Visit

Subjects should perform the Study Completion Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last.

For subjects who terminate from the study early (refer to Section 9.3.3), the Early Termination Visit (ETV) will occur 4 weeks after the final dose of study drug or after

withdrawal from study participation, whichever occurs last. Subjects who terminate from study drug early should continue to perform the remaining visit assessments in Section 12.3 as applicable until study completion.

Any AE that caused a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the ETV will be followed by the Investigator.

Every reasonable effort should be made to contact any subject who is lost to follow-up.

The following study activities will be performed at the ETV:

- Physical examination
- Vital signs
- 12-lead ECG
- Clinical laboratory tests
- Chest x-ray
- Sedimentation rate
- Urine pregnancy test, if applicable (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.). For safety reasons, this test must be performed prior to the chest X-ray.
- Assessment of AEs
- Concomitant medications
- Diet query
- 3-day diet diary (It is preferable that the subject complete the diary 3 days immediately prior to the Early Termination Visit.)
- PK sample
- Blood Phe and plasma tyrosine concentration
- Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, anti-rAvPAL-PEG neutralizing antibodies)

13 DATA QUALITY ASSURANCE

BioMarin personnel or designees will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol and other regulatory document requirements, any applicable randomization procedures, source document requirements, CRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, a CRA will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on CRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting and drug accountability records.

The designated clinical data management group will enter or transfer CRF data into a study database.

Data quality control and analysis will be performed by BioMarin or a designee, based on a predefined analysis plan.

14 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

14.1 Statistical and Analytical Plans

Because of the small sample size, no formal statistical tests will be performed. Categorical data will be summarized using frequency and percent, while continuous data will be summarized using descriptive statistics (ie, number of observations, mean, median, standard deviation [SD], minimum, and maximum).

The statistical analysis plan (SAP) will provide additional details about the planned statistical analysis.

14.2 Safety Analysis

All subjects who receive any amount of study drug in this study and have postdose safety information will be included in the safety analyses.

The Medical Dictionary for Regulatory Activities terminology (MedDRA) will be used by the sponsor to assign system organ class and preferred term classification to events and diseases based on the original terms entered on the Case Report Form (CRF).

The incidence of AEs will be summarized by system organ class, preferred term, relationship to study drug, and severity. A by–subject listing will be provided for those subjects who experience a serious AE (SAE), including death, or experience an AE associated with early withdrawal from the study or study drug. Hypersensitivity AEs and AEs that result in dosing interruption or dose level reduction are of interest, and the percentage of subjects who report these AEs will be presented.

Clinical laboratory data will be summarized by the type of laboratory test. Frequency and percentage of subjects who experience abnormal (ie, outside of reference range) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory test. For each clinical laboratory test, descriptive statistics will be provided for baseline and all subsequent post-treatment visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics of vital signs, physical examination results, ECG results, and X-ray results, and immunogenicity test results will also be provided. Additionally, antibodies and titers will be summarized by scheduled time point.

14.3 Pharmacokinetic Analysis

Subjects who complete the Substudy will have the following PK parameters assessed: area under the plasma concentration-time curve (AUC), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half life ($t_{1/2}$), and clearance (CL/F). Should

data become available from previous rAvPAL-PEG studies that indicate study drug accumulation should also be measured, additional blood draws may be added for PK analysis as needed per the discretion of the Investigator in consultation with the sponsor's medical monitor.

14.3.1 Pharmacokinetic Substudy Analysis

For subjects who completed the Substudy, the steady-state PK of rAvPAL-PEG in subjects who have achieved and maintained target blood Phe will be explored. This substudy has been completed as of 20 December 2013.

14.4 Efficacy Analysis

Data from all subjects who receive any amount of study drug and who have any post-treatment efficacy data will be included in the efficacy analysis.

Blood Phe concentration at each scheduled time point will be summarized using descriptive statistics (mean, SD, median, minimum, and maximum). Change in blood Phe concentration from baseline (to be defined in the SAP) to each scheduled time point and presence/absence of antibodies will also be summarized. In addition, the proportion of subjects who have maintained target Phe concentrations of 60-600 µmol/L will be summarized by each scheduled time point.

The relationship of dietary Phe intake (per information reported on the subject diet diary) and blood Phe concentration will also be explored. Subjects who received any amount of study drug with any post-treatment blood Phe concentration measurements and diet diary information will be included in this exploratory analysis.

Details regarding exploratory analyses will be provided in the Statistical Analysis Plan.

14.5 Determination of Sample Size

Subjects who participated in previous rAvPAL-PEG studies may be enrolled into this study. No formal sample size calculation was conducted for this study or the PAL-003 Substudy.

14.6 Handling of Missing Data

All analyses will be presented based on observed data only. Missing data will not be imputed for analyses unless sensitivity analyses are warranted. If imputations are used, detailed imputation methods will be described in the SAP before locking the database.

14.7 Interim Analyses

Safety will be assessed throughout the study on an ongoing basis (refer to Section 15 for information regarding the DMC).

14.8 Changes in the Conduct of the Study or Planned Analyses

Only BioMarin may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with BioMarin, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the Investigator should inform BioMarin and the full IRB within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by BioMarin and the IRB, and all active subjects must again provide informed consent.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued and the SAP will prevail.

15 DATA MONITORING COMMITTEE (DMC)

The DMC will act independently in an advisory capacity to BioMarin to monitor the safety of rAvPAL-PEG in subjects who participate in the study.

The responsibilities of the DMC are to:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring.
- Evaluate the progress of the trial, subjects' risk versus benefit, and other factors that could affect the study outcome.
- Assess the effect and relevance of new external evidence.
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study.
- Protect the safety of the study participants in accordance with the stopping criteria as defined in study protocol Section 9.1.2.
- Make recommendations to the BioMarin concerning continuation or termination of the study or other study modifications based on observations.

16 COMPENSATION, INSURANCE, AND INDEMNITY

There will be no charge to study subjects to be in this study. BioMarin will pay all costs of tests, procedures, and treatments that are part of this study. In addition, after IRB approval, BioMarin may reimburse the cost of travel for study-related visits. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort, whether or not related to the study subject's disease, that are not part of this study. Costs associated with hospitalizations, tests, and treatments should be billed and collected in the way that such costs are usually billed and collected.

The Investigator should contact BioMarin immediately upon notification that a study subject has been injured by the IP or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the Investigator to seek medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries.
The treating physician should bill the subject's health insurance company or other third party payer for the cost of this medical treatment, unless the executed Clinical Trial Agreement between BioMarin and the institution of the investigator specifies otherwise. If the subject has followed the Investigator's instructions, BioMarin may pay for reasonable and necessary medical services to treat the injuries caused by the IP or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries without prior recourse to third party payer billing. If this is the case, BioMarin will comply with the law.

17 CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic case report forms will be provided for each subject. The Investigator must review and electronically sign the completed CRF casebook to verify its accuracy.

CRFs must be completed using a web–based application that has been validated. Study site personnel will be trained on the application and will enter the clinical data from source documentation. Unless explicitly allowed in the CRF instructions, blank data fields are not acceptable.

In the event of an entry error, or if new information becomes available, the site personnel will correct the value by deselecting the erroneous response and then selecting or entering the factual response. In compliance with 21 CFR Part 11, the system will require the site personnel to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

BioMarin's policy is that study data on the CRFs must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must therefore agree to allow direct access to all source data. Subjects (or their legally authorized representative) must also allow access to their medical records, and subjects will be informed of this and will confirm their agreement when giving informed consent.

A CRA designated by BioMarin will compare the CRFs with the original source documents at the study site and evaluate them for completeness and accuracy before designating them as "source data verified" (SDV). If an error is discovered at any time or a clarification is needed, the CRA, or designee, will create an electronic query on the associated field. Site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re–query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

Before a subject's CRF casebook can be locked, all data fields must be SDV and all queries closed. The Data Manager, or designee, will then set the status of the forms, visits, and the entire casebook to locked. The investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. Upon completion of the clinical study report for the entire study, an electronic copy of each site's casebooks will be copied to a compact disk (CD) and sent to each site for retention with other study documents.

18 STUDY MONITORING AND AUDITING

Qualified individuals designated by BioMarin will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The PI agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records, of the study subjects, and, if requested, agrees to assist the monitors. The PI and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

Members of BioMarin's GCP Compliance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify BioMarin immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files.

19 RETENTION OF RECORDS

The PI must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The PI must consult a BioMarin representative before disposal of any study records, and must notify BioMarin of any change in the location, disposition or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced, including paper copies of study records (e.g., subject charts) as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the U.S. or an ICH region and until (1) there are no pending or contemplated marketing applications in the United States or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a BioMarin agreement. BioMarin must be notified and will assist with retention should the PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the PI/institution as to when these documents no longer need to be retained.

20 USE OF INFORMATION AND PUBLICATION

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Trial Agreement between BioMarin and the Investigator/Institution. Consideration for authorship of all publications will be based on compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals ("Uniform Requirements") of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

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22 INVESTIGATOR RESPONSIBILITIES

22.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He or she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He or she will personally conduct or supervise the study.
- He or she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He or she has read and understands the information in the Investigator's Brochure, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He or she will ensure that adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.
- He or she will ensure that the IRB complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB. Additionally, he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

23 SIGNATURE PAGE

Protocol Title: Long-term Extension of a Phase 2, Open-Label Dose-Finding Study to Evaluate the Safety, Efficacy, and Tolerability of Multiple Subcutaneous Doses of rAvPAL-PEG in Subjects with PKU

Protocol Number: PAL-003, Amendment 8

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

[redacted] 31 March 2017
Date

Printed name: [redacted]

24 PROTOCOL AMENDMENT TEXT REVISIONS

The following is a summary of significant protocol revisions; added text is underlined and deleted text is ~~struck~~. Additional administrative changes have been made for consistency and clarity throughout this amendment and are reflected in the protocol body. Revisions made to the Synopsis (Section 2) are reflected in the corresponding protocol section as appropriate. Revisions to cross references to protocol tables, figures, sections, and references reside within the protocol body.

Section	Revision	Rationale for Change
Global change	~~Up to 102~~ Approximately 119 weeks	9
Global change	Premedication with H1 antagonist, H2 antagonist, and ~~non-steroidal anti-inflammatory medication (NSAIDs)~~antipyretics (eg, acetaminophen) approximately 2-3 hours prior to study drug should be administered for 1 week upon reintroduction of study drug at Part 3, Week 1 and Part 4, Week 1, as well as upon reintroduction of study drug after resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in Part 4. If non-steroidal anti-inflammatory medication (NSAIDs) is administered as a premedication, it ~~NSAIDs~~ should be given with food ~~and may be omitted if not tolerated~~.	6
Section 7.4.1, Toxicity Due to Exposure to Polyethylene Glycol (PEG)	Based on phase 3 data, as of 4MAR2016, there have been no reported events of glomerulonephritis or renal failure reported with pegvaliase administration. Of the 10 subjects identified with UAC ratios ≥ 3 mg/mmol on 3 or more consecutive measurements, values were generally in the microalbuminuria range and none had concurrent sustained hematuria. Seven of these 10 subjects had microalbuminuria at baseline prior to pegvaliase exposure, of which 2 events resolved while on pegvaliase treatment and 4 events did not progress while subjects remained on pegvaliase. One of these 7 subjects with baseline microalbuminuria (3.62 mg/mmol at baseline) had an increase in their UAC ratio during the study, which was more apparent after dose titration to 60 mg of pegvaliase (remaining in microalbuminuria range, with a most recent value of 20.36 mg/mmol), however evaluating relationship to pegvaliase treatment is confounded	1

Section	Revision	Rationale for Change
	by a medical history of obesity, hypertension, and new onset pre-diabetes during the Phase 3 study that are conditions also associated with development of microalbuminuria. The subject continues on pegvaliase treatment with continued safety monitoring. Three of the 10 subjects developed new onset microalbuminuria for at least 3 consecutive measurements during the Phase 3 study, one of which resolved while continuing on pegvaliase treatment and 1 subject continues to have fluctuating low level microalbuminuria with intermittent values in the normal range. One subject discontinued from the Phase 3 study after a few intermittent elevations into the microalbuminuria range. The subject had a maximum UAC ratio of 175 mg/g on Day 8 on the study; at the time of study and study drug discontinuation (Day 36), UAC ratio was within the normal range at 19 mg/g. The final study visit recorded for this subject (Day 67) had a UAC ratio of 17 mg/g. This subject reported a single concurrent grade 1 event of urticaria, which resolved in 2 days, and no other reported events suggestive of immune complex disease. None of the subjects described above have developed sustained elevations in serum creatinine or concurrent sustained hematuria. To monitor for renal toxicity, subjects participating in this study will undergo urinalysis, will be assessed for urine albumin/creatinine ratio, and will undergo blood chemistry tests to assess renal function. ~~In this study, subjects will be monitored closely with urine examinations and blood chemistries to follow kidney function.~~	
Section 9.1, Overall Study Design and Plan	A subject will continue in PAL-003 until one of the following occurs: • The subject has completed the study through the ~~Month 98~~Week 476 visit.	9

Section	Revision	Rationale for Change
Section 9.1, Overall Study Design and Plan	~~Because the risks of taking BMN 165 during pregnancy and breastfeeding are unknown, subjects cannot take BMN 165 if they are trying to conceive, are pregnant, or are breastfeeding. Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study will be discontinued immediately from study drug. Male subjects who have impregnated a female partner may re-start study drug after conception but must return to the study-required contraception use, which must include one barrier method. During Part 4 only, subjects who remain in the study after discontinuation of study drug due to pregnancy may re-introduce BMN 165 dosing after the birth has been reported (or termination of the pregnancy) and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting BMN 165 dosing must be per investigator and sponsor agreement based on an assessment of the known risks and potential benefits while taking BMN 165. Female subjects must return to the study-required contraception use, which must include one barrier method, immediately after the birth (or termination of the pregnancy).~~ Because the risks of taking BMN 165 during pregnancy and breastfeeding are unknown, subjects cannot take BMN 165 if they are trying to conceive, are pregnant, or are breastfeeding (refer to Section 9.3.2 for Exclusion Criteria). Male subjects who are planning to impregnate a female partner and female subjects who are trying to become pregnant during the study must be temporarily discontinued from study drug for 4 weeks prior to trying to conceive. During that time, subjects must use two acceptable methods of contraception. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician. Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests. Subjects who are pregnant or are trying to conceive and have temporarily discontinued study drug should not perform the scheduled PK assessments. Male subjects who have impregnated a female partner may re-start study drug after conception following the investigator's consultation with and approval by the medical monitor. Male subjects must return to the study-required contraception use (refer to Section 9.3.1 for Inclusion Criteria), which must include one barrier method, prior to restarting study drug. Female subjects who remain in the study after temporary discontinuation of study drug due to pregnancy may restart BMN 165 dosing after a confirmed negative urine pregnancy test result, the birth has been reported (or termination of the pregnancy), and breastfeeding has completed (if applicable) or after the subject is no longer actively trying to conceive. Re-starting BMN 165 dosing requires the investigator's prior consultation with and approval by the medical monitor. Female subjects must return to the study-required contraception use (refer to Section 9.3.1), which must include one barrier method, immediately after the birth (or termination of the pregnancy).	2

Section	Revision	Rationale for Change
Section 9.1, Table 9.1.1, Schedule of Events	The Schedule of Event tables have been updated to reflect the changes in this amendment. Additionally, the monthly visits have been modified to occur every 8 weeks.	8, 11
Section 9.1.1, Response to Hypersensitivity Adverse Events	Subjects who experience an injection-site skin reaction that lasts ≥ 14 days should be referred to a dermatologist for consultation and a skin biopsy.	5
Section 9.6, Dietary or Other Protocol Restrictions	Subjects will be instructed that diet should not be altered during the course of the study. Any decision to modify subject diet must be made per the Investigator and must be performed under the supervision of the Investigator. Subjects who are planning to become pregnant (or impregnate a female partner) may modify their diet in consultation with the investigator and/or study dietician.	2
Section 9.7.5.1, Pregnancy Testing	Female subjects of childbearing potential will have a urine pregnancy test at the time points specified in the Schedules of Events (Table 9.1.1). Subjects who are confirmed to be pregnant per a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.	2
Section 9.7.5.2, Antibody Testing	BioMarin ~~or CRO~~ will perform all antibody testing, except for IgE antibodies, which will be assessed by a Contract Research Organization.	11
Section 9.7.6, Clinical Laboratory Assessments	Any abnormal test results determined to be clinically significant by the investigator should be repeated (at the discretion of the investigator) until the cause of the abnormality is determined, the value returns to the level from Day 1 of Part 2 or to within normal limits, or the investigator determines that the abnormal value is no longer clinically significant. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation	1, 4

Section	Revision	Rationale for Change
	if results were within normal range at baseline. Subjects who had elevated results at baseline followed by a confirmed subsequent increase of 100-200 mg/g from baseline should also be referred to a nephrologist for consultation.	
Section 9.7.6.1, Table 9.7.6.1.1 (Clinical Laboratory Tests)	The table has been revised to add the following footnotes: [a] To be performed by central laboratory. If urine pregnancy test is positive or equivocal, serum sample will be taken and assessed by a central laboratory. It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. [c] It is recommended that urine samples are obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at baseline. Subjects who had elevated results at baseline followed by a confirmed subsequent increase of 100-200 mg/g from baseline should also be referred to a nephrologist for consultation.	1, 4
Section 9.7.7, Exploratory Sample Analyses	Blood and urine samples will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. For each portion of the blood and urine samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study, unused sample portions may be used during the study or after the subject's study completion for assay development or other purposes.	7

Section	Revision	Rationale for Change
	All samples collected in this study may be used for exploratory research. In addition, samples collected for other purposes may be used for exploratory research use once the primary use has been completed.	
Section 10.1., Adverse Events	Factors suggestive of a causal relationship could include (but are not limited to) the following: • Plausible temporal relationship • Absence of alternative explanations • Rarity of event in a given patient or disease state • Absence of event prior to study drug exposure • Consistency with study product pharmacology • Known relationship to underlying mechanism of study drug action • Similarity to adverse reactions seen with related drug products • Abatement of AE with discontinuation of study drug, and/or recurrence of AE with reintroduction of study drug	3
Section 10.3, Adverse Events Occurring Secondary to Other Events	In general, AEs occurring secondary to other events (eg, cascade events) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the eCRF. However, medically important events that may be linked and/or separated in time should be recorded as independent events on the eCRF. For example, if severe hemorrhage leads to renal failure, both events should be recorded separately on the eCRF.	3

Section	Revision	Rationale for Change
Section 10.4, Persistent or Recurrent Adverse Events	A persistent AE is one that extends continuously, without resolution, between subject evaluation time points. Such an event should be recorded only once on the eCRF unless its severity increases or decreases (in which case it should be recorded again on the AE eCRF). A recurrent AE is one that occurs and resolves between subject evaluation time points, but then subsequently recurs. All recurrences of the AE should be individually recorded as a separate event on the AE eCRF.	3
Section 10.5, Abnormal Laboratory Values	Laboratory test results will be recorded on the laboratory results pages of the eCRF, or appear on electronically produced laboratory reports submitted directly from the central laboratory, if applicable. Any laboratory result abnormality fulfilling the criteria for a SAE should be reported as such, in addition to being recorded as an AE in the eCRF. A clinical laboratory abnormality should be documented as AE if it is not otherwise refuted by a repeat test to confirm the abnormality and any one or more of the following conditions is met: • Accompanied by clinical symptoms • Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation) • Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment). • The abnormality suggests a disease and/or organ toxicity	3

Section	Revision	Rationale for Change
	• The abnormality is of a degree that requires active management (eg, change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation, etc.) This applies to any protocol and non-protocol specified safety and efficacy laboratory result from tests performed after the first dose of study medication that falls outside the laboratory reference range and meets the clinical significance criteria. This does not apply to any abnormal laboratory result that falls outside the laboratory reference range but that does not meet the clinical significance criteria (these will be analyzed and reported as laboratory abnormalities), those that are considered AEs of the type explicitly exempted by the protocol, or those which are a result of an AE that has already been reported.	
Section 10.6, Pre-existing Conditions	A pre-existing condition is one that is present at the start of the study. Such conditions should be recorded as medical history on the appropriate eCRF. A pre-existing condition should be recorded as an AE or SAE during the study only if the frequency, intensity, or character of the condition worsens during the study period. It is important to convey the concept that a pre-existing condition has changed by including applicable language in the verbatim description of the event (eg, more frequent headaches).	3
Section 10.7, General Physical Examination Findings	At screening, any clinically significant abnormality should be recorded as a pre-existing condition (refer to Section 10.6). During the study, any new clinically significant findings and/or abnormalities discovered on physical examination that meet the definition of an AE (or an SAE) must be recorded and document as an AE or SAE on the AE eCRF.	3

Section	Revision	Rationale for Change
Section 10.8, Hospitalization, Prolonged Hospitalization, or Surgery	Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol (refer to Section 10.2). There are some hospitalization scenarios that do not require reporting as an SAE when there is no occurrence of an AE. These scenarios include planned hospitalizations or prolonged hospitalizations to the following: • Perform a protocol-mandated efficacy measurement • Undergo a diagnostic or elective surgical procedure for a pre-existing medical condition that has not changed • Receive scheduled therapy (study drug or otherwise) for the study indication	3
Section 10.9, Deaths	All deaths that occur during the AE reporting period (refer to Section 10.1 for nonserious AEs and Section 10.2 for SAEs), regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE. When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.	3
Section 10.10, Pregnancy	Pregnancy in a subject or partner should be reported to BPV within 24 hours of the site becoming aware of the pregnancy by faxing the Pregnancy Form in the study reference materials. The reporting period for pregnancies begins with informed consent and continues until 4 weeks following the last study drug dose or the End of Treatment visit. In addition, pregnancy in a subject should also be reported on the End of Study eCRF and the Subject Disposition eCRF if the subject withdraws from the study due to pregnancy….	3

Section	Revision	Rationale for Change
	Abortion, whether therapeutic or spontaneous, should always be classified as an SAE (as the Sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the Sponsor as an SAE.	
Section 10.11, Follow-up of Subjects after Adverse Events	For some SAEs and AESIs, the sponsor may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details (eg, hospital discharge summary, consultant report, or autopsy report) deemed necessary to appropriately evaluate the SAE report.	3
Section 10.12, Post-Study Adverse Events	At the last scheduled visit, the investigator should instruct each subject to report to the investigator and/or to BPV directly any subsequent SAEs that the subject's personal physician(s) believes might be related to prior study treatment. The investigator should notify the study sponsor of any death or SAE occurring at any time after a subject has discontinued or terminated study participation if the investigator believes that the death or SAE may have been related to prior study treatment. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject who participated in this study.	3
Section 10.14, BioMarin Pharmacovigilance Contact Information	Name: [redacted] Address: 105 Digital Drive Novato, CA 94949 USA Phone: [redacted] Fax: [redacted] E-mail: [redacted]	10

Section	Revision	Rationale for Change
Section 12.3, Treatment Period	During the Treatment Period, additional visits may occur if deemed necessary to monitor AEs or blood Phe concentrations. On days that a dose is given, assessments will be done predose unless otherwise specified. Subjects must have AEs and concomitant medications assessed whenever dose is administered even if the administration does not coincide with a scheduled clinic visit. If there is a skin reaction that lasts ≥ 14 days, the Skin Reaction eCRF should be completed and the subject should be referred to a dermatologist for consultation and a skin biopsy. It is recommended that a~~A~~ photograph of the skin reaction ~~may~~ be taken by the subject or the site to help assess the event; photographs ~~and~~ may be collected by the sponsor.	5
Section 12.3.2, Weekly Telephone Visit	Subjects should have a weekly telephone visit conducted. Subjects must still come into the clinic for the scheduled ~~monthly~~ visits every 8 weeks per Table 9.1.1. The following should be discussed with the subject during the weekly telephone visit:	8
Section 12.3.4, Every 8 Weeks	~~Beginning with Week 4, monthly v~~Visits must be performed in the clinic every 8 weeks. The following study activities will be performed: • Physical examination • Sedimentation rate • Complement C_3 and C_4 • PK sample • Serum anti-rAvPAL-PEG antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and anti-rAvPAL-PEG neutralizing antibodies) • Prefilled syringe refresher training (every 24 weeks only) • If additional training is needed on days subsequent to the ~~monthly~~ 8-week scheduled visit, additional clinic visit(s) or HHRN may be used.	8

Section	Revision	Rationale for Change
Section 12.3.5, Quarterly Visits	This section has been deleted.	8
Section 17, Case Report Forms and Source Documents	Before a CRF casebook can be locked, data fields must be source data verified and queries closed. ~~The investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete.~~ The Data Manager (or designee) will then set the status of the forms, visits, and the entire casebook to Locked. The investigator will then electronically sign the casebook, specifying that the information on the CRFs is accurate and complete. Upon completion of the clinical study report, an electronic copy of each site's casebooks will be copied to a compact disk and will be sent to each site for retention with other study documents.	11