Official Title: A Phase 3b Open-Label Study to Evaluate the Effect of Kuvan® on Neurocognitive Function, Maintenance of Blood Phenylalanine Concentrations, Safety, and Population Pharmacokinetics in Young Children with Phenylketonuria

NCT Number: NCT00838435

Applicant/MAH: BioMarin Pharmaceutical Inc.

Version Date: 30 November 2010

CLINICAL STUDY PROTOCOL

Study Title:	A Phase 3b Open-Label Study to Evaluate the Effect of Kuvan® on Neurocognitive Function, Maintenance of Blood Phenylalanine Concentrations, Safety, and Population Pharmacokinetics in Young Children with Phenylketonuria
Protocol Number:	PKU-015
Investigational Product:	Kuvan® (sapropterin dihydrochloride; 6R-BH4)
IND/EUDRACT Number:	069708
Indication:	Phenylketonuria (PKU)
Sponsor:	BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Development Phase:	Phase 3b
Sponsor's Responsible Medical Officer:	[redacted], MD BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949
Duration:	7 years
Dose:	20 mg/kg/day
Patient Population:	Children age 0 to 6 years
Date of Original Protocol:	21May08
Date of Amendment 1	25Nov08
Date of Amendment 2	**30Nov10**

CLINICAL STUDY PROTOCOL AMENDMENT SUMMARY

Amendment: 2

Date: 30 November 2010

RATIONALE AND SUMMARY OF CHANGES

The following change was made in accordance with a recommendation by regulatory authorities:

1. Exclusion Criteria will include any use of a phosphodiesterase type 5 inhibitor (PDE5 inhibitor) by any study subject.

The following changes were made in the protocol for consistency and clarification between protocol sections and training documents provided to the study sites.

2. Clarifications for the administration of neurocognitive testing age ranges and frequency of administration were changed within the protocol to match the training manual.
3. Dietary changes for blood Phe results < 120 µmol/L and > 240 µmol/L are clarified.
4. The "Study Schematic" was modified to reflect that Substudy 1 is from Week 0 though Month 6 of the 7-year study.
5. "Weight" was separated from the growth assessments and was added to all study visits, including Week 0. Height and head circumference were removed from Week 0 when the Week 0 visit is≤7 days from the screening visit.
6. Ages were modified for the population PK samples to take into account closing of the 0 to 1 year old age group due to a higher than expected responder rate. The number of subjects undergoing PK sampling was decreased from 30 subjects to 10 subjects in the 0 to 1 year age group and increased from the first 50 subjects to the first 70 subjects in the>1 to 6 year age group.
7. Blood sampling timing was clarified.
8. Administration, dose rounding, and daily dosing of investigational product (IP) was clarified.
9. The Summary of Risks section was updated to correspond to the risks in Investigator Brochure (version 5).
10. The Medical Monitor was updated to Dr [redacted], MD.

11. The Product Characteristics and Labeling section was modified to reflect the color change due to a change in compaction process.
12. Clinical laboratory clarifications include the addition of chloride as part of the electrolyte evaluation in the chemistry panel. Urinalysis may be performed in the clinical lab or via "dipstick" in children too young to provide a urine sample.
13. Emphasis was added that every subject should complete a termination visit, which should occur on the date that the subject withdraws from or completes this study.
14. Instructions for the 3-Month Interim phone contact performed between the clinic visits that occur after Month 6 month and through Month 84 were clarified. Assessments include weight, adverse events/serious adverse events and concomitant medications, and dispensing of study drug.
15. Clarification was made to study procedures regarding procedure time points.
16. Abbreviations updated to match use in protocol.

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.
1/Title Page	**Sponsor's Responsible Medical Officer:** [redacted] ~~, MD~~ [redacted] ~~, Clinical Affairs~~ [redacted], MD BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949	Dr [redacted] has replaced Dr. [redacted] as the Sponsor's Responsible Medical Officer	10
2/Synopsis: Study Design and Plan	… However, if a subject's blood Phe concentration drops below 120 µmol/L, at the discretion of the investigator, a gradual increase of approximately 5 to 20 mg/kg of daily dietary Phe intake may be added to the diet. It is important to avoid unstable swings in the blood Phe values ~~increased by 5 mg/kg initially and may be increased further at the discretion of the Investigator if the subject's blood Phe concentration remains below 120 µmol/L~~. ……	Dietary changes clarified	3

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td>2/Synopsis: Study Design and Plan</td><td>Part 2: Neurocognitive Study

Neurocognitive Assessments

<table>
<tr><td>Age Group</td><td>Infancy</td><td colspan="2">Preschool</td><td>School-Age</td></tr>
<tr><td>Assessment</td><td>Bayley-III</td><td>WPPSI-III</td><td>WPPSI-III</td><td>WISC-IV</td></tr>
<tr><td>Age</td><td>0 months through < 30 months</td><td>≥ 30 months through < 4 years</td><td>≥ 4 years through < 7 years</td><td>≥ 7 years and older</td></tr>
<tr><td>Frequency</td><td>Every 6 months ± 2 weeks</td><td>Once a year ± 2 weeks</td><td>Once a year ± 2 weeks</td><td>Every 2 years ± 2 weeks</td></tr>
</table>

~~Neurocognitive measurements in subjects 0-2 years old will be made every six months using the Bayley-III until subjects reach age 30 months. After age 30 months, WPPSI-III will be administered until subjects reach age 6 years. Subject ≥ 6 years old will be tested using WISC-IV. Neurocognitive testing will be conducted using WPPSI-III and WISC-IV every 12 months until the subject reaches age 7, then every 2 years through the end of the study.~~

The Bayley-III will be administered to subjects who are from 0 months to less than 30 months of age and will be administered every 6 months ± 2 weeks. At 30 months old or older, the subjects will be administered the WPPSI-III. The WPPSI-III has two forms. The younger children will be administered the first form for ages</td><td>Table added and reworded for clarity</td><td>2</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
	30 months to less than 4 years old. The second form will be used for subjects 4 years of age to less than 7 years old. The WPPSI-III will be administered yearly ± 2 weeks. Beginning at 7 years old, study subjects will be administered the WISC-IV every 2 years ± 2 weeks. Growth, blood Phe concentration, and safety will be monitored. These and the neurocognitive assessments ,,,		
2/Synopsis: Study Design and Plan	**Substudy 2: Population Pharmacokinetics (PK) in Subjects 0-6 Years Old** For the population PK evaluation, plasma samples from the first ~~30~~ 10 subjects enrolled in Part 1 who are 0-1 years old and the first ~~50~~ 70 subjects ~~who~~ in the >1-6 years age group (3 samples each from subjects ≤ 1 year old; 4 samples each from subjects >1 years old) will be collected…	Because of greater than expected response rate in the under 1 year age group the demographics were changed	6
2/Synopsis: Diagnosis and All Criteria for Inclusion and Exclusion	• Use of phosphodiesterase type 5 inhibitor, often shortened to PDE5 inhibitor (eg.sildenafil citrate, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil)	Added to Exclusion criteria per recommendation by regulatory authorities	1
2/Synopsis: Investigational Product(s), Dose, Route, and Regimen	Kuvan is provided in tablets of 100 mg of sapropterin dihydrochloride. A dose of 20 mg/kg will be administered dissolved in water, ~~or~~ apple juice, or soft foods, based on subject's age and ability, and taken orally once daily with food.	Soft foods added for ease of administration.	8

Section No./Title	Revision	Comments	Relates to Change No.
Substudy 1: Safety and Efficacy in Subjects 0-6 Years Old	… Kuvan dose may also be reduced after week 5 and after consultation with the Medical Monitor if the subject does not tolerate the 20 mg/kg/day dose…	Clarification of dose changes after Week 5	8
2/Synopsis: Criteria for Evaluation	**Efficacy:** Neurocognitive development will be evaluated based on IQ measured by the age-appropriate tests listed below: • Bayley-III in ages 0 to ≤ 30 months • WPPSI-III in ages ≥ 30 months to ≤ 7 years ~~≤ 6 years~~ • WISC-IV in ages ≥ 7 years ~~> 6 years~~	Clarification of ages	2

Section No./Title	Revision	Comments	Relates to Change No.
2.1/Schedule of Events	~~**Table 2.1: Schedule of Event, All Subjects**~~ ~~**Part 1: Evaluation of Kuvan Responsiveness[a]**~~ | ~~**Part 2: Long-term Assessment of Neurocognitive Ability and Safety[b]**~~ | ~~**Substudy 1: 6-Month Safety and Efficacy[c]**~~ | ~~**Follow-up/ETV[d]**~~	**From Amendment 1:** **Schedule of Event,** Heading of Table 2.1 changed.	15
2.1/Schedule of Events	**Table 2.1: Schedule of Event, All Subjects** **Part 1: Evaluation of Kuvan Responsiveness (includes Substudy 2)[a]** | **Part 2: Long-term Assessment of Neurocognitive Ability and Safety[b]** **Substudy 1: 6-Month Safety and Efficacy** | **Follow-up/ETV[d]**	From Amendment 2: Substudy 1: Changed from Months 1 to 6 (Amendment 1) to Week 0 to Month 6 (Amendment 2)	15

Section No./Title	Revision	Comments	Relates to Change No.
2.1/Schedule of Events	Height, ~~weight~~, head circumference: X X X X X X X X X X X X X X X X X (X[i])	Weight deleted from height, weight and head circumference	5
2.1/Schedule of Events	Weight: X X X X X X X X X X X X X X X X X X X X (X[i])	Weight added to every visit as a separate row.	5
2.1/Schedule of Events	Bayley-III[q] (ages 0 to ≤30 months) WPPSI-III[r] (≥ 30 months to < 7 years) ~~≤ 6 years~~) WISC-IV[s] (≥ 7 years) ~~(> 6 years)~~	Clarification of age ranges for assessments	2
2.1/Schedule of Events	[c] Monthly visits should occur within 5 days of scheduled timepoint (ie, ± 5 days) for M2, M3, M4, M5 and M6. All visits will occur at the clinic. [d] ~~Follow-up will be performed for all subjects, even if they withdraw early from the study. Subjects will be contacted by telephone 4 weeks (28 ± 3 days) after the last dose of study drug, unless required to return for a clinic visit (see footnotes i, q, r, s, and t).~~	Clarification	2,3,5,6,12,14, 15

Section No./Title	Revision	Comments	Relates to Change No.
	[d] Every subject should complete a Follow-up or Termination visit to end their participation in the study. Follow-up visit is performed for subjects who complete only Part 1 of the study, or who are enrolled into Part 2 and complete Part 2. The Termination visit is completed by subjects who withdraw or are discontinued from the study. Subjects will be contacted by telephone 4 weeks (28 ± 3 days) after the last dose of study drug, unless required to return for a clinic visit (see footnotes i, q, r, s, and t). [e] All study procedures for the W 0 visit must occur before the first dose of study drug is taken during that visit. Subjects will receive their first dose of study drug under observation after all other procedures have been performed. Physical examination, clinical laboratory tests, height~~, weight,~~ and head circumference measurements will be repeated only if the W 0 visit is > 7 days after the Screening visit. [e] Superscript "e" added to WO and removed from height and head circumference, physical examination, and laboratory test assessments in the Week O comumn. [i] Superscript "i" removed from neurocognitive assessments at Follow –up/ETV [j] Clinical laboratory tests include hematology, chemistry, and urinalysis. Urinalysis may be performed in the clinical lab or via "dipstick" in children too young to provide a urine sample. [o] If a subject's blood Phe concentration drops below 120 µmol/L, at the discretion of the investigator, the subject's dietary Phe restriction ~~will~~ may be modified to allow a gradual increased intake of approximately 5-20 mg/kg of Phe per day, avoiding unstable swings in the blood Phe values..		

Section No./Title	Revision	Comments	Relates to Change No.
	[q] Bayley-III, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects and at ~~every~~ 6 months intervals thereafter until the subject is ≥ 30 months old. At that time, the subject will be assessed using the WPPSI-III test. If a subject leaves the study between 4 to 6 months after the preceding Bayley-III assessment, this test, or the WPPSI-III test if the subject is ~~older than~~ ≥30 months at dropout, will be done at follow-up. [r] WPPSI-III, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects, then yearly thereafter until the subject is ≥7 years old ~~> 6 years old~~. At that time, the subject will be assessed using the WISC-IV test. If a subject leaves the study between 9 months and 1 year after the preceding WPPSI-III assessment, this, or the WISC-IV test if the subject is older than 7 years at dropout, will be done at follow-up. [s] The WPPSI-III has two forms. The younger children will be administered the first form for ages 30 months up to 4 years old. The second form will be used for subjects 4 years of age up to 7 years old. [ts] WISC-IV, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects and every 2 years ~~yearly~~ thereafter. If a subject leaves the study between 9 months and 1 year after the preceding WISC-VI assessment, this test will be done at follow-up. [u] The WPPSI-III or WISC-IV will continue to be administered on an annual or bi-annual basis, dependent on subject's age, at months, 60, 72 and 84. [u] Superscript "u" added to WISC-IV neurocognitive assessment in the M60/M84 column.		

Section No./Title	Revision	Comments	Relates to Change No.
	[v] 3-Month interim phone contact should occur within 1 week of scheduled timepoint (ie, ± 1 week). Phone calls will be performed between clinic visits that occur after month 6 and through Month 84. Subjects will be contacted by telephone to document the subject's weight, study drug supply, AEs, and Con Meds. [v] Superscript "v" removed from Height, head circumference and added to the Part 2: Long-term Assessment of Neurocognitive Ability and Safety. [w] The population PK study will be performed using 80 subjects, with at least ~~30~~ 10 subjects 0-1 years old, and ~~50~~ 70 subjects > 1-6 years old who enroll in Part 1 of the study. See Section 12.3 for details.		
4/List of Abbreviations and Definitions of Terms	PDE5 Phosphodiesterase type 5 inhibitor	Abbreviations updated to match use in protocol	16
7.4.1/Summary of Risks	~~The most serious adverse reactions during Kuvan administration (regardless of relationship to treatment) were gastritis, spinal cord injury, streptococcal infection, testicular carcinoma, and urinary tract infection. Mild to moderate neutropenia was noted during Kuvan administration in 24 of 579 subjects (4%). The most common (≥4% of subjects treated with Kuvan) across all studies (n = 579) were headache, diarrhea, abdominal pain, upper respiratory tract infection, pharyngolaryngeal pain, vomiting, and nausea.~~ Adverse reactions have usually been mild and transient. The integrated safety database consists of 579 patients in Kuvan clinical trials. These patients were treated at daily doses of 5 mg/kg, 10 mg/kg, or 20 mg/kg, from 8 days to over	Changed adverse reaction paragraph from text in the package insert to text from the Investigator's Brochure	9

Section No./Title	Revision	Comments	Relates to Change No.
	6 months. No dose-related or extent of exposure-related change in frequency of AEs was observed. Six serious adverse events occurred in clinical trials, none were considered to be study drug related. One subject withdrew from a clinical trial due to an AE of pregnancy. The most frequent AEs thought to be related to Kuvan in placebo-controlled trials were pharyngolaryngeal pain, and rhinorrhoea. The most frequently reported adverse reactions in all clinical trials (> 4%), regardless of relationship to study drug, were headache, diarrhea, abdominal pain, upper respiratory tract infection, pharyngolaryngeal pain, vomiting, and nausea. Mild to moderate neutropenia was noted during Kuvan administration in 24 of 579 subjects (4%) and in 3 of 59 subjects receiving placebo (5%).		
9.1/Overall Study Design and Plan	Figure 9.1.1	Pediatric Safety and Efficacy clarified	4
9.1/Overall Study Design and Plan	Part 1: Evaluation of Kuvan Responsiveness Beginning at Week 0…However, if a subject's blood Phe concentration drops below 120 µmol/L, at the discretion of the investigator, a gradual increase of approximately 5 to 20 mg/kg of daily dietary Phe intake may be added to the diet. It is important to avoid unstable swings in the blood Phe values ~~increased by 5 mg/kg initially and may be increased further at the discretion of the Investigator if the subject's blood Phe concentration remains below 120 µmol/L~~. ….	Dietary changes clarified	3

Section No./Title	Revision	Comments	Relates to Change No.
9.1/Overall Study Design and Plan	Part 2: Neurocognitive Study Subjects who are responsive to Kuvan (see the definition of responsiveness in Inclusion and Exclusion ~~Eligibility~~ Criterion) will… The types of neurocognitive tests used (ie, Bayley Scale of Infant and Toddler Development® Third Edition [Bayley-III], Wechsler Preschool and Primary Scale of Intelligence™ Third Edition [WPPSI III], and/or Wechsler Intelligence Scale for Children® Fourth Edition [WISC IV]), will be those considered appropriate for the subject's age. ~~Neurocognitive measurements in subjects 0-2 years old will be made every six months using the Bayley-III until subjects reach age 30 months. After age 30 months, WPPSI-III will be administered until subjects reach age 7 6 years. Subjects ≥ 7 years old > 6 years old will be tested using WISC-IV. Neurocognitive testing will be conducted using WPPSI-III and WISC-IV every 12 months until the subject reaches age 7, then the WISC-IV every 2 years yearly through the end of the study. Neurocognitive measurements in subjects 0-2 years old will be made every 6 months using the Bayley-III until subjects reach age 30 months. After age 30 months, WPPSI-III will be administered until subjects reach age 7 years. Subjects ≥ 7 years old will be tested using WISC-IV. Neurocognitive testing will be conducted using WPPSI-III every 12 months until the subject reaches age 7, then the WISC-IV every 2 years through the end of the study. Growth, blood Phe concentration, and safety will be monitored. These assessments will be performed every 6 to 24 months, depending on the type of assessment, until the end of the study. Refer to Table 2.1 for details.~~	Clarification of age ranges for assessments	2

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

Table 9.1.1 Neurocognitive Assessments

Age Group	Infancy	Preschool		School-Age
Assessment	Bayley-III	WPPSI-III	WPPSI-III	WISC-IV
Age	0 months through < 30 months	≥ 30 months through < 4 years	≥ 4 years through < 7 years	≥ 7 years and older
Frequency	Every 6 months ± 2 weeks	Once a year ± 2 weeks	Once a year ± 2 weeks	Every 2 years ± 2 weeks

The Bayley-III will be administered to subjects who are from 0 months to 30 months of age and will be administered every 6 months ± 2 weeks. At 30 months old or older, the subjects will be administered the WPPSI-III. The WPPSI-III has two forms. The younger children will be administered the first form for ages 30 months to 4 years old. The second form will be used for subjects 4 years of age to 7 years old. The WPPSI-III will be administered yearly ± 2 weeks. Beginning at 7 years old study, subjects will be administered the WISC IV every 2 years ± 2 weeks.

Growth, blood Phe concentration, and safety will be monitored. These assessments will be performed every 6 to 24 months, depending on the type of assessment, until the end of the study. Refer to Table 2.1 for details.

Section No./Title	Revision	Comments	Relates to Change No.
	The study will be considered complete when approximately 45 subjects have been followed for 7 years and have at least 2 post-treatment WPPSI III and/or WISC IV tests administered 2 years apart. After study completion, subjects will be given the opportunity to participate in a registry study that will follow longer term outcomes.		
Substudy 1: Safety and Efficacy in Subjects 0-6 Years Old	Kuvan dose may also be reduced **after week 5 and** after consultation with the Medical Monitor if the subject does not tolerate the approximately 5-20 mg/kg/day dose.	Clarification of when the dose may be changed.	8
9.1/Overall Study Design and Plan	For the population PK evaluation, the first ~~30~~ 10 subjects in the 0-1 year age group and the first ~~50~~ 70 subjects in the > 1-6 year age group ….	Because of greater than expected response rate in the under 1 year age group the demographics were changed	6
9.2/Discussion of Study Design, Including Choice of Control Group	…During Substudy 1 ~~(Months 2 – 6),~~ safety and efficacy assessments will be conducted weekly during Week 0 to Week 4, then monthly for Months 2 through Month 6.	Clarification of procedures	15

Section No./Title	Revision	Comments	Relates to Change No.
9.3.2/Exclusion Criteria	• Use of phosphodiesterase type 5 inhibitor, often shortened to PDE5 inhibitor (eg.sildenafil citrate, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil)	Added to Exclusion criteria per recommendation by regulatory authorities	1
9.4.1/Treatments Administered	In Part 1, beginning at Week 0, subjects will receive Kuvan at a dose of 20 mg/kg/day orally, provided in tablets that each contain 100 mg of sapropterin, for a total of 4 weeks. ~~All study drug will be taken in the dissolved form throughout the study~~. For ease in administration and instruction to the families, the dose can also be rounded up or down: For children who are older and can take their medication from a cup or glass, rounding of the dose to the nearest whole tablet is acceptable. For example, a child weighing 11 kg would have a dose of 220 mg of Kuvan which that may be rounded down to 200 mg (2 tablets). A child weighing 14 kg would have a dose of 280 mg that may be rounded up to 300 mg (3 tablets). Whether the dose is determined using Table 9.4.4.1 or by rounding the dosage up or down, be consistent with the method. This is especially important through Week 4 when responsiveness is being determined. The first dose in Part 1 will be taken at the Week 0 visit under observation, after the completion of all other study procedures. The remaining doses in Part 1 will be taken daily ~~at the same time with the morning meal~~ with food to increase absorption, preferably at the same time each day. The Kuvan dose cannot be adjusted during Part 1 without consultation with the Medical Monitor. Subjects who are enrolled into Part 2 will initially continue to take 20 mg/kg/day Kuvan ~~with the morning meal~~ at the same time each day with a meal. After week 5, at the discretion of the Investigator and after consultation with the Medical Monitor, a	Dose administration and rounding clarified	8

Section No./Title	Revision	Comments	Relates to Change No.
	subject's Kuvan dose may be lowered if the subject does not tolerate the 20 mg/kg/day dose.		
9.4.2.1/Product Characteristics and Labeling	…Tablets are round, ranging from off white to light yellow, light orange/pink, beige, or mottled (speckled) appearance, and debossed with "177".	Tablet color clarified	11
9.4.4/ Directions for Administration	Kuvan (sapropterin dihydrochloride) tablets should be administered orally ~~at the morning meal~~ with food to increase absorption, preferably at the same time each day with a meal. Kuvan tablets can ~~should~~ be dissolved in 4 to 8 oz. (120 to 240 mL) of water or apple juice … all of the medicine is taken. Administration of crushed Kuvan tablets may also be stirred in soft foods such as apple sauce or lemon pudding. A missed dose… At the discretion of the Investigator, tablets may be dissolved in as little as 5 mL water or apple juice per tablet, based on the subject's size and capability. For infants weighing < 5 kg, a single Kuvan tablet … of this Kuvan solution. A dosing table for subjects weighing ~~20~~ 10 kg and less is shown below (Table 9.4.4.1).	Soft foods added for ease of administration.	8

<table>
<tr><th>Section No./Title</th><th>Revision</th><th>Comments</th><th>Relates to Change No.</th></tr>
<tr><td>9.4.4/ Directions for Administration</td><td>Table 9.4.4.1: Kuvan Dosing Table for Young Children
<table>
<tr><th>Child's Weight (kg)</th><th>Dose Given (Milligrams)</th><th>mL of Solution</th><th>No. of 100 mg Tablets Dissolved</th><th>mL Administered to Subject</th></tr>
<tr><td>1</td><td>20</td><td>5</td><td>1</td><td>1</td></tr>
<tr><td>2</td><td>40</td><td>5</td><td>1</td><td>2</td></tr>
<tr><td>3</td><td>60</td><td>5</td><td>1</td><td>3</td></tr>
<tr><td>4</td><td>80</td><td>5</td><td>1</td><td>4</td></tr>
<tr><td>5</td><td>100</td><td>5</td><td>1</td><td>5</td></tr>
<tr><td>6</td><td>120</td><td>10</td><td>2</td><td>6</td></tr>
<tr><td>7</td><td>140</td><td>10</td><td>2</td><td>7</td></tr>
<tr><td>8</td><td>160</td><td>10</td><td>2</td><td>8</td></tr>
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<tr><td>10</td><td>200</td><td>10</td><td>2</td><td>10</td></tr>
<tr><td>~~11~~</td><td>~~220~~</td><td>~~15~~</td><td>~~3~~</td><td>~~11~~</td></tr>
<tr><td>~~12~~</td><td>~~240~~</td><td>~~15~~</td><td>~~3~~</td><td>~~12~~</td></tr>
</table></td><td>Dose administration clarification</td><td>8</td></tr>
</table>

Section No./Title	Revision	Comments	Relates to Change No.
	~~13~~ ~~260~~ ~~15~~ ~~3~~ ~~13~~ ~~14~~ ~~280~~ ~~15~~ ~~3~~ ~~14~~ ~~15~~ ~~300~~ ~~15~~ ~~3~~ ~~15~~ ~~16~~ ~~320~~ ~~20~~ 4 ~~16~~ ~~17~~ ~~340~~ ~~20~~ ~~4~~ ~~17~~ ~~18~~ ~~360~~ ~~20~~ ~~4~~ ~~18~~ ~~19~~ ~~380~~ ~~20~~ ~~4~~ ~~19~~ ~~20~~ ~~400~~ ~~20~~ ~~4~~ ~~20~~		
9.4.6.1/Selection of Timing of Dose for Each Subject	Taking the daily dose ~~in the morning~~ at the same time of day with a meal may help to facilitate treatment compliance.	Dose administration clarification	8

Section No./Title	Revision	Comments	Relates to Change No.
9.6/Dietary or Other Protocol Restrictions	Subjects ~~and their parent(s) or guardian(s)~~ must be willing to adhere to a prescribed Phe restricted diet in order to maintain blood Phe concentrations within the recommended ranges established at the subject's study site. The goal for blood Phe concentration during the study is ≤ 240 µmol/L. Following standard of care, all subjects and their parent(s) or guardian(s) will meet with a study dietician ~~nutritionist~~ at each visit to review dietary Phe intake. A 3 day dietary record…	Clarification	3
9.6/Dietary or Other Protocol Restrictions	If blood Phe levels fall below 120 µmol/L, dietary Phe may be added … be monitored closely. Increases in Phe supplement will be based on blood Phe level, age, and ideal body weight for a subject's gender, weight, and height. • If a subject's blood Phe falls below 120 µmol/L, at the discretion of the investigator, a gradual increase of approximately 5-20 mg/kg of Phe supplement may be added to the diet. It is important to avoid unstable swings in the blood Phe values • If a subject's blood Phe level goes above 240 µmol/L, dietary Phe ~~will~~ may be decreased by approximately 5 to 20 mg/kg or defined by the standard used at each treatment center, at the discretion of the investigator.	Dietary changes clarified	3
9.7.1.2/ Physical Examination Findings	Complete physical examinations will include the evaluation of all major body systems, height, weight, and head circumference. Other body systems may be examined. ~~Day 1~~ Week 1 of Part 1 results will be the baseline values, and clinically significant changes from baseline will be recorded as an AE.	Clarification of procedures	15

Section No./Title	Revision	Comments	Relates to Change No.
9.7.1.4/Blood Phe Control	Samples for blood Phe concentration should be drawn simultaneously with samples drawn for PK on days when both types of blood draw are required. With the exception of ~~Day 1~~ the PK samples (Part 1), blood for Phe concentration measurements …in the Schedule of Events (Table 2.1).	Clarification	7
9.7.2 /Primary Efficacy Variable	Neurocognitive testing will be performed within 6 weeks of determination of Kuvan responsiveness in all Kuvan-responsive subjects. Subsequent neurocognitive measurements will be obtained every 6 months until the subject is ~~>~~ ≥ 30 months old…	Clarification or age groups for neurocognitive testing	2
9.7.3/ Secondary Efficacy Variables	The first secondary efficacy endpoint is change in developmental growth factors, as measured by height, ~~weight,~~ and head circumference from baseline. These measurements will be made at Screening, Week 0, Week 4, Month 3, Month 6, and every 6 months thereafter~~.~~, while the weight will be measured at every visit. The second secondary efficacy variable is the baseline and 6-month follow up neurocognitive testing results using Bayley-III in children age 0 **to less than** 30 months old.	Weight deleted from height, weight and head circumference	2, 5
Table 9.7.4.1.1: Clinical Laboratory Tests	Chloride Urinalysis may be performed in the clinical lab or via "dipstick" in children too young to provide a urine sample	Chloride was added as part of the electrolyte evaluation in the chemistry panel. Urine testing was clarified.	12

Section No./Title	Revision	Comments	Relates to Change No.
11.1 / Neurocognitive Assessments	The Bayley-III is a tool for assessing all facets of development in infants within an age range of 12 months up to 30 months, with normative data… The WPPSI-III is a tool for assessing the intelligence of children ~~2 years 6 months through~~ ≥ 30 months through ≤7 years 3 months old. The WPPSI-III is administered on two forms as age appropriate.… The WISC-IV assesses the intelligence of children ~~6~~ 7 years 0 months through 16 years 11 months old. …	Clarification of are groups for assessment	2
12.1.2/Screening Visit	• … • Weight • Height~~, weight,~~ and head circumference • … • Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)	Weight was separated from height and head circumference and added to every visit	5,

Section No./Title	Revision	Comments	Relates to Change No.
12.1.3.1/Week 0 ~~Day 1~~ Visit	Within 28 days of completing screening assessments, the following study activities will be performed on ~~Day 1 (~~Week 0~~)~~ of Part 1. Assessments will be done predose unless otherwise specified: • Weight • ~~Height, weight, and head circumferences~~ • Dietary review with ~~nutritionist~~ dietician • ~~Physical examination if > 7 days after Screening~~ • ~~Clinical laboratory tests if > 7 days after Screening~~ • Blood Phe concentration ~~if > 7 days after Screening~~ . … • Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) • Physical examination, clinical laboratory tests, height and head circumference measurements will be repeated only if the Week 0 visit is > 7 days after the Screening visit • Dispense Study Drug	Weight was separated from height and head circumference and added to every visit and visit procedure clarification	5,15

Section No./Title	Revision	Comments	Relates to Change No.
12.1.3.2/Week 1 through Week 4 Visits (± 2 days)	• Weight • … • Dietary review with ~~nutritionist~~ Phe modification (if necessary) with dietician	Weight added dietary review clarified	5, 15
12.1.3.4/Week 4 Visit Only (± 2 days)	Week 4 Visit ~~o~~Only (± 2 days) • Physical examination ~~(Week 4 only)~~ • Height, ~~weight~~, and head circumference ~~(Week 4 only)~~ • Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential ~~(Week 4 only)~~ (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result)	Title change and clarification of visits	5, 15

Section No./Title	Revision	Comments	Relates to Change No.
12.1.4.1/~~Month 2-3~~ **Month 2-5 (±5 days)**	• Vital signs • Weight • Clinical laboratory tests • Blood Phe concentration • Concomitant medications • Assessment of AEs • Dietary review with Phe modification (if necessary) with a dietician • Dispense Study Drug	Title changed and procedures clarified	15
~~12.1.4.1~~ /~~Month 2-3 (± 5 days);~~ 12.1.4.2 **Month 2/Visit Only (±5days)**	• Administration of age-appropriate IQ test; this test must be completed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects who continue into the Neurocognitive Study	Changed section number and title	15
~~12.1.4.2 Month 3 (± 5 days)~~ **12.1.4.3/ Month 3 and 6 Visits Only (±5days)**	• Height and head circumference • Physical examination	Changed section number and title and procedures clarified	15
12.1.4.4/Month 6 Visit Only (± 5days)	• Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy	Section added with Title and procedure clarification	15

Section No./Title	Revision	Comments	Relates to Change No.
	test result.) • 6-month repeat testing with Bayley-III in children 0-2 years old at enrollment		
~~12.1.4.3/ 6 Month Visits, Starting at Month 6 (Months 6, 12,18, etc. ± 2 weeks~~ **12.1.4.5** **Months 6, 12, 18, 24, 30, etc. (6 month intervals ± 2 weeks)**	• Weight • Height~~, weight,~~ and head circumference • Dietary review with ~~nutritionist~~ Phe modification (if necessary) with a dietician … • Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) • Blood tryptophan and tyrosine ~~Month 6 and 12, then yearly~~ (starting at Month 12) • Neurocognitive tests ~~with Bayley-III until the subject reaches age 30 months~~ (see Section 2.1 Summary of Events and Section 9 Investigational Plan)	Changed section number and title and procedures clarified	5, 15
~~12.1.4.4 Yearly Visits (Months 12, 24, 36, 48, etc. ± 2 weeks)~~	~~• Vital signs~~ ~~• Blood Phe concentration~~ ~~• Height, weight, and head circumference~~	Changed section number and title and visit procedures clarified	15

Section No./Title	Revision	Comments	Relates to Change No.
12.1.4.6/Months 12, 24, 36, 48, 60, 72 and 84 (annual intervals ± 2 weeks)	~~• Physical examination~~ ~~• Clinical laboratory tests~~ ~~• Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential~~ ~~• Concomitant medications~~ ~~• Dietary review with nutritionist~~ ~~• Assessment of AEs~~ • ~~Dispense Study Drug~~ • Blood tryptophan, ~~and~~ tyrosine ~~(Starting at Month 12)~~ • ~~Neurocognitive tests using WPPSI-III or WISC-IV (starting at Month 12; performed every 12 months until the subject reaches age 7, then every 2 years through the end of the study.)~~ •		

Section No./Title	Revision	Comments	Relates to Change No.
12.1.4.7 3-Month Interim Phone Contact (Months 9, 15, 21, 27, etc, ± 1 week)	• Weight • Assessment of AEs • Concomitant Medications • Dispensing Study Drug	Section added. Clarification of visit procedures	5,15
12.1.5 Early Termination and Follow up Visit(s) (within 28 +/- 3 days of last dose of study drug)	**Every subject should complete either a Follow-up or** Termination Visit, to end their participation in the study. The Follow-up Visit is completed by a subject who completes only Part 1 of the study, or who is enrolled into Part 2 and completes Part 2. The Termination Visit is completed by a subject who withdraws or is discontinued from the study. The Follow-up or Termination Visit should occur 28 days after the subject's final dose of drug. Every reasonable effort will be made to have the subject complete a Follow-up or Termination Visit. If the subject does not complete a Follow-up or Termination Visit in the clinic, minimally a follow-up phone call will be made. • Dietary review with ~~nutritionist (if visit is required)~~ dietician • Physical examination ~~(if visit is required)~~ (if at a clinic visit) • Vital signs ~~(if visit is required)~~ (if at a clinic visit) • Weight • Height, ~~weight~~ and head circumference ~~(if visit is required)~~ (if at a clinic visit) • Clinical laboratory tests ~~(if visit is required)~~ (if at a clinic visit)	Clarification of visit procedures	13, 15

Section No./Title	Revision	Comments	Relates to Change No.
	• Blood tryptophan and tyrosine ~~(if visit is required)~~ (if at a clinic visit) • Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result. (if at a clinic visit) • Neurocognitive test using Bayley-III if the follow up or early termination visit is 4-6 months after the last assessment. • Neurocognitive tests using WPPSI III or WISC IV if the follow-up or early termination visit is 9 months or more after the last assessment (if at a clinic visit)…		
12.2.2 ~~Monthly Visits (~~Months 2 - 6 (± 5 Days)	• Weight • Dietary review with ~~nutritionist~~ Phe modification (if necessary) with a dietician	Weight added and clarification of visit procedures	5,15
12.2.3 Months 3 and 6 Only (± 5 Days)	• Height~~, weight,~~ and head circumference	Title modified and weight removed from Height, weight, and head circumference.	15
12.2.4 Month 6 Only ~~(± 30 days)~~ (± 2 weeks)	• Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)	Title modified and clarification of visit procedures	15
12.2.5 Early	Section deleted	Early Termination	NA

Section No./Title	Revision	Comments	Relates to Change No.
Termination from Substudy 1 (within 28 +/- 3 days of last dose of study drug)		section covered in Section 12.1.5	
12.3/ Substudy 2: Population Pharmacokinetic Study in Subjects 0 6 Years Old	A population PK study will be completed in a total of 80 subjects. Of these 80 subjects, 10 ~~30~~ will be ages 0 to ≤ 1 years and 70 ~~50~~ will be > 1 to 6 years old. Subjects will take their dose ~~in the morning~~at the same time of day with a meal, per site instructions, to obtain samples for the population PK analysis within the specified time windows (eg, before dosing and 0.22 to 8 hours or 7 to 480 minutes, after dosing). The exact times of dosing will be recorded. Blood samples will ~~still need to~~ be collected approximately 2.5 to 5 hours after ~~breakfast~~ the meal to obtain blood Phe concentrations while in Phase 1 of this study (Week 0-Week 6)….	Clarification of PK age range and blood sample timing	6,7

2 SYNOPSIS

NAME OF COMPANY BioMarin Pharmaceutical Inc. 105 Digital Drive Novato, CA 94949 NAME OF FINISHED PRODUCT: Kuvan® NAME OF ACTIVE INGREDIENT: sapropterin dihydrochloride; 6R-BH4	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

A Phase 3b Open-Label Study to Evaluate the Effect of Kuvan® on Neurocognitive Function, Maintenance of Blood Phenylalanine Concentrations, Safety, and Population Pharmacokinetics in Young Children with Phenylketonuria

PROTOCOL NUMBER:

PKU-015

STUDY SITES:

Up to 40 sites

PHASE OF DEVELOPMENT:

3b

STUDY RATIONALE:

Rigorous control of diet is typically advocated in children 4 years and under with phenylketonuria (PKU) because brain sensitivity to high phenylalanine (Phe) concentration is expected to be greatest during these years of rapid neurocognitive development. Among PKU patients, the strongest determinant of intelligence quotient (IQ) and cognitive function is compliance with blood Phe control. Several clinical studies with Kuvan have already demonstrated efficacy in reducing blood Phe in subjects older than 4 years. This study will examine whether use of Kuvan at an earlier age preserves neurocognitive function and normal growth, as well as providing long-term safety data and efficacy data for controlling blood Phe concentrations within acceptable ranges. In addition, 2 substudies will be conducted. Substudy 1 will provide 6 months of safety, efficacy, and neurocognitive data in subjects 0-6 years old. Substudy 2 will provide pharmacokinetic data in children 0-6 years old.

OBJECTIVES:

Primary Objective

- To evaluate the long-term efficacy of Kuvan in preserving neurocognitive function in children with PKU when treatment is initiated at 0-6 years

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Secondary Objectives

- To evaluate the long-term safety of Kuvan in the study population
- To evaluate the effect of Kuvan on growth parameters in the study population

Substudy 1: 6-Month Safety and Efficacy in Children 0-6 Years Old

Primary Objective

- To evaluate the safety of 6 months of treatment with Kuvan in children with PKU who are 0-6 years old.

Secondary Objective

- To evaluate the efficacy of 6 months of treatment with Kuvan in controlling blood Phe concentration within acceptable ranges in children with PKU who are 0-6 years old
- To evaluate baseline neurocognitive function for all Kuvan-responsive subjects and 6-month Bayley-III data for subjects who are 0-2 years old at enrollment

Substudy 2: Population Pharmacokinetics in Children 0-6 Years Old

Primary Objective

- To evaluate the population pharmacokinetics of Kuvan in young children

STUDY DESIGN AND PLAN:

Part 1: Evaluation of Kuvan Responsiveness

Beginning at Week 0 and after all baseline assessments are completed, subjects enrolled into the study will receive 20 mg/kg Kuvan daily for 4 weeks. Subjects will return to the clinic weekly for safety assessments and blood Phe concentration measurement. Subjects should continue with their current level of dietary Phe intake. However, if a subject's blood Phe concentration drops below 120 µmol/L, **at the discretion of the investigator, a gradual increase of approximately 5 to 20 mg/kg of Phe supplement may be added to the diet. It is important to avoid unstable swings in the blood Phe values.** No dose adjustments can be made during Part 1 without consultation with the Medical Monitor.

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Part 2: Neurocognitive Study

Subjects who are responsive to Kuvan (see the definition of responsiveness in Eligibility Criterion) will continue to receive daily single doses of 20 mg/kg Kuvan orally. At the discretion of the Investigator, **a gradual increase of approximately 5 to 20 mg/kg of Phe supplement may be added to the diet. It is important to avoid unstable swings in the blood Phe values**. Kuvan dose may also be reduced after Week 5 after consultation with the Medical Monitor, in subjects who do not tolerate the 20 mg/kg/day dose. Neurocognitive testing will be performed within 6 weeks of determination of Kuvan responsiveness in all Kuvan-responsive subjects. The types of neurocognitive tests used (ie, Bayley Scales of Infant and Toddler Development®-Third Edition [Bayley-III®], Wechsler Preschool and Primary Scale of Intelligence™-Third Edition [WPPSI™-III], and/or Wechsler Intelligence Scale for Children®-Fourth Edition [WISC®-IV]), will be those considered appropriate for the subject's age.

Neurocognitive Assessments

Age Group	Infancy	Preschool		School-Age
Assessment	Bayley-III	WPPSI-III	WPPSI-III	WISC-IV
Age	0 months through < 30 months	≥ 30 months through < 4 years	≥ 4 years through < 7 years	≥ 7 years and older
Frequency	Every 6 months ± 2 weeks	Once a year ± 2 weeks	Once a year ± 2 weeks	Every 2 years ± 2 weeks

The Bayley-III will be administered to subjects who are from 0 months to less than 30 months of age and will be administered every 6 months ± 2 weeks. At 30 months old or older, the subjects will be administered the WPPSI-III. The WPPSI-III has two forms. The younger children will be administered the first form for ages 30 months to less than 4 years old. The second form will be used for subjects 4 years of age to less than 7 years old. The WPPSI-III will be administered yearly ± 2 weeks. Beginning at 7 years old, study subjects will be administered the WISC-IV every 2 years ± 2 weeks.

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Growth, blood Phe concentration, and safety will be monitored. These **and the neurocognitive** assessments will be performed every 6 to 24 months, depending on the type of assessment, until the end of the study. Refer to Table 2.1 for details.

The study will be considered complete when approximately 45 subjects have been followed for 7 years and have at least 2 post-treatment WPPSI-III and/or WISC-IV tests administered 2 years apart.

After study completion, subjects will be given the opportunity to participate in a registry study that will follow longer-term outcomes.

Substudy 1: Safety and Efficacy in Subjects 0-6 Years Old

All Kuvan-responsive subjects will be enrolled in Substudy 1 and will be assessed monthly for the first 6 months of the study. Blood Phe concentrations, various measures of growth, and safety data will be collected. The subject's daily Phe intake will be increased only if the subject's blood Phe levels fall below 120 µmol/L. Kuvan dose may also be reduced **after week 5 and** after consultation with the Medical Monitor if the subject does not tolerate the 20 mg/kg/day dose. Refer to Table 2.1, Schedule of Events for the timing of assessments.

Substudy 2: Population Pharmacokinetics (PK) in Subjects 0-6 Years Old

For the population PK evaluation, plasma samples from the first 10 subjects enrolled in Part 1 who are 0-1 years old and the first - **70** subjects **in the >1-6 years age group** (3 samples each from subjects ≤ 1 year old; 4 samples each from subjects >1 years old) will be collected at the Week 0 through Week 4 visits according to a predetermined schedule. See Section 12.3 and Table 12.3.1 for sampling windows. In addition, investigators will be asked to collect a blood sample for total biopterin testing at the time of an SAE, when possible, if it is considered by the investigator to be probably or possibly related to Kuvan.

NUMBER OF SUBJECTS PLANNED:

The study is expected to comprise a minimum of 60 Kuvan-responsive subjects. At least 20 subjects will be 0-2 years old and at least 10 of these 20 subjects will be 0-1 years old. At least 20 subjects will be 2-4 years old and the remaining subjects will be 4-6 years old. In order to obtain a minimum of 60 Kuvan-responsive subjects, we anticipate that more than 230 subjects will be enrolled in Part 1, Evaluation of Kuvan Responsiveness.

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:

Individuals eligible to participate in Part 1, Evaluation of Kuvan Responsiveness and Substudy 2 (population PK) must meet all of the following criteria:

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- Established diagnosis of PKU with hyperphenylalaninemia (HPA) documented in the medical record by at least 2 blood Phe concentrations ≥ 360 µmol/L (6 mg/dL) taken at least 3 days apart
- Documented blood Phe control (defined by the standard used at each treatment center) prior to study enrollment, if applicable (eg, the subject is old enough for these data to be collected); blood Phe concentrations for subjects < 6 months old at Screening must be considered controlled and stable by the Investigator
- Willing to adhere to a prescribed Phe-restricted diet in order to maintain blood Phe concentrations within the recommended ranges established at the subject's study site
- Age 0 to 6 years old, inclusive, at Screening
- Parent(s) or guardian(s) willing and able to provide written, signed informed consent after the nature of the study has been explained, and prior to any research-related procedures
- Parent(s) or guardian(s) willing and able to comply with all study procedures
- Female subjects of childbearing potential (as determined by the investigator) and sexually mature male subjects willing to use a medically accepted method of contraception throughout the study. Female subjects of childbearing potential willing to undergo periodic pregnancy tests during the course of the study

Individuals eligible to participate in Part 2: Neurocognitive Study and Substudy 1 must meet all of the following criteria:

- Completion of Week 4 visit in Part 1
- Responsive to Kuvan during Part 1, defined as a ≥ 30% average reduction in blood Phe concentration calculated as the mean of the weekly percent change from baseline in blood Phe concentration at Weeks 1, 2, 3, and 4
- Bayley-III or IQ test score ≥ 80 within 6 weeks of determination of Kuvan responsiveness

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

- Established diagnosis of primary tetrahydrobiopterin (BH4) deficiency
- Known hypersensitivity to Kuvan or its excipients

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- History of organ transplantation
- Perceived to be unreliable or unavailable for study participation or to have parents or legal guardians who are perceived to be unreliable or unavailable
- Use of methotrexate or other medications that inhibit folate metabolism
- Serious neuropsychiatric illness (eg, major depression) not currently under medical control
- Use of Kuvan or any investigational agent within 30 days prior to Screening, or known requirement for any investigational agent prior to completion of all scheduled study assessments
- Concurrent disease or condition that would interfere with study participation or safety (eg, seizure disorder, oral steroid-dependent asthma or other condition requiring oral or parenteral corticosteroid administration, insulin-dependent diabetes
- **Use of phosphodiesterase type 5 inhibitor, often shortened to PDE5 inhibitor (eg. sildenafil citrate, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil)**
- Any condition that, in the view of the Principal Investigator (PI), renders the subject at high risk for failure to comply with treatment or to complete the study

INVESTIGATIONAL PRODUCT(S), DOSE, ROUTE AND REGIMEN:

Kuvan is provided in tablets of 100 mg of sapropterin dihydrochloride. A dose of 20 mg/kg will be administered dissolved in water, apple juice, or soft foods, based on subject's age and ability, and taken orally once daily with food.

DURATION OF TREATMENT:

Evaluation of Kuvan Responsiveness: 4 weeks

Neurocognitive Study: 7 years

Substudy 1: 6 months

Substudy 2: 4 weeks

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REFERENCE THERAPY(IES), DOSE, ROUTE AND REGIMEN:

Not applicable

CRITERIA FOR EVALUATION:

Efficacy:

Neurocognitive development will be evaluated based on IQ measured by the age-appropriate tests listed below:

- Bayley-III in ages 0 to < 30 months
- WPPSI-III in ages ≥ 30 months to <7 years
- WISC-IV in ages ≥ 7 years

The long-term benefit of Kuvan treatment will be evaluated by demonstrating that neurocognitive development is not worse than the expected development for subjects with PKU on a Phe-restricted diet.

Growth will be assessed using the following measurements:

- Height, weight, and head circumference

Safety:

Safety assessments will include medical history, adverse events (AEs), vital signs, physical examinations including height, weight, and head circumference, and clinical laboratory tests (chemistry, hematology, and urinalysis).

Substudy 1: Safety and Efficacy in Children Age 0-6 Years

Efficacy:

The benefit of Kuvan treatment will be evaluated by determining whether Kuvan can control blood Phe concentrations within acceptable ranges for age and based on the criteria used at each treatment center. The assessment will be made using the proportion of subjects whose blood Phe concentration is within each subject's acceptable range and the mean change in blood Phe concentration from baseline at each timepoint.

Safety:

Safety assessments will include medical history, AEs, vital signs, physical examinations including height, weight, and head circumference, blood tryptophan and tyrosine concentration, standard clinical

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laboratory tests (chemistry, hematology, and urinalysis), and an ECG (electrocardiogram), if required.

Substudy 2: Population PK Study in Children Age 0-6 Years

The pharmacokinetics of Kuvan will be assessed through measurement of total biopterin concentration in plasma samples obtained during the Week 0 through Week 4 visits using a population pharmacokinetics approach.

STATISTICAL METHODS:

Sample Size

The calculation of sample size assumes that each child will have 2 post-treatment test results from the WPPSI-III and/or WISC-IV tests at least 2 years apart. The goal is to exclude a mean 2.5-point loss per year in score. Given a 2-sided Type I error rate of 0.05, if the mean IQ score at baseline is 100, the standard deviation is 15, and the correlation between the 2 test results is 0.8, a sample size of 45 subjects will yield 90% power.

To account for the possibility of dropouts and to have reasonable precision for estimating change in IQ from the time a child reaches at least age 30 months until the end of the study, a minimum of 60 Kuvan-responsive subjects will be enrolled in the study.

Primary Efficacy Endpoint and Analysis

The primary efficacy endpoint is the IQ score measured by the WPPSI-III or WISC-IV tests. Analysis of neurocognitive development using the IQ scores will be performed using a repeated measures model. The outcome variable will be the IQ score from WPPSI-III and/or WISC-IV tests. The slope of the test scores will be calculated. The treatment will be considered successful if the lower confidence limit of the mean change in test score over a 2-year period excludes a decline of greater than 5 points. All data will be included in a combined analysis, stratified by testing sequence used (WPPSI-III/WPPSI-III, WPPSI-III/WISC-IV, or WISC-IV/WISC-IV).

Secondary Efficacy Endpoint and Analysis

The secondary efficacy endpoint is:

- Change in developmental growth factors, measured by height, weight, and head circumference from baseline

This secondary endpoint will be analyzed using a longitudinal model.

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Safety Analysis

The original terms reported in case report forms (CRFs) to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent 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 had an SAE, including death, or had an AE resulting in early discontinuation of study drug.

Laboratory data will be summarized by the type of laboratory test. The frequency and percentage of subjects who experience an abnormality (ie, outside of reference ranges) or clinically significant abnormality after study drug administration will be presented for each laboratory analyte. For each laboratory analyte, descriptive statistics will be provided for values measured at each scheduled visit, and for change in values from baseline to each scheduled visit. Similarly, descriptive statistics of vital signs will be provided.

Interim safety analyses will be conducted annually through completion of the study.

Substudy 1: Safety and Efficacy Analyses

Efficacy will be assessed by the ability of Kuvan, administered over a 6-month period, to control blood Phe concentrations within acceptable ranges for age and for the criteria used at the treatment centers. Efficacy endpoints are:

- Proportion of subjects whose blood Phe concentration is within acceptable ranges at post-treatment visits
- Mean change in blood Phe concentration from baseline to post-treatment monthly visits

An exact 95% CI for the proportion of subjects whose blood Phe concentration within acceptable ranges at each post-treatment visit and a 95% confidence interval (CI) for the mean change in blood Phe concentration from baseline to post-treatment monthly visits will be provided for the efficacy assessment. Baseline blood Phe concentration will be calculated using the mean of Screening and Week 0 (prior to dosing with Kuvan) results.

Safety results for the first 6 months of treatment will be analyzed as described above.

Substudy 2: Pharmacokinetic Analyses

The time course of the calculated BH4 concentration (from plasma total biopterin measurement) will be modeled to estimate the following population PK parameters for Kuvan: apparent clearance (CL/F) with associated inter-individual variability, apparent volume of distribution (Vc/F) with associated

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inter-individual variability, and absorption rate constant with associated inter-individual variability (Ka). Data from the Substudy 2 may also be pooled with PK data obtained from adult subjects in previous clinical studies.		

2.1 Schedule of Events

Table 2.1: Schedule of Events, All Subjects

Assessment	Screening[a]	Part 1: Evaluation of Kuvan Responsiveness (includes Substudy 2)[a]				Part 2: Long-term Assessment of Neurocognitive Ability and Safety[b,v]															Follow-up/ETV[d]
		Substudy 1: 6-Month Safety and Efficacy																			
		W0[e]	W1	W2	W3	W4[f]	M2[c]	M3[c]	M4[c]	M5[c]	M6[c]	M12[g]	M18[g]	M24[g]	M30[g]	M36[g]	M42[g]	M48[g]	M54[g]	M60/ M84[g]	
Informed consent	X																				
Medical history	X																				
Vital signs	X	X[h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X[i])
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X[i])
Height, head circumference[v]	X	X				X		X			X	X	X	X	X	X	X	X	X	X	(X[i])
Physical examination	X	X				X		X			X	X	X	X	X	X	X	X	X	X	(X[i])
Laboratory tests[j]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X[i])
ECG[k]		X																			
Pregnancy tests[l]	X	X				X					X	X	X	X	X	X	X	X	X	X	X
Blood Phe[m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Tryptophan, tyrosine	X					X		X			X	X		X		X		X		X	(X[i])
Concomitant meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X[n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Treatment with Kuvan/Dispense Study Drug		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Dietary Phe Rx and dietary Phe intake	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Definitions: Bayley-III, Bayley Scales of Infant and Toddler Development, Third Edition; ETV, Early Termination Visit; M, Month; Phe, phenylalanine; Rx, prescription; W, Week; WISC-IV, Wechsler Intelligence Scale for Children, Fourth Edition; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence, Third Edition.

Table 2.1: Schedule of Events, All Subjects, cont'd

Assessment	Screening	Part 1: Evaluation of Kuvan Responsiveness (includes Substudy 2)[a]					Part 2: Long-term Assessment of Neurocognitive Ability and Safety[b,v]														
		Substudy 1: 6-month Safety and Efficacy																			Follow-up/ETV[d]
		W0[e]	W1	W2	W3	W4[f]	M2[c]	M3[c]	M4[c]	M5[c]	M6[c]	M12[g]	M18[g]	M24[g]	M30[g]	M36[g]	M42[g]	M48[g]	M54[g]	M60/ M84[g]	
Dietary Phe modification[o]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Neurocognitive tests[p]																					($X^{q,r,s,t}$)
Bayley-III[q] (ages 0 to <30 months)							X				X	X	X	X							(X^{q})
WPPSI-III[r,s] (≥30 months to < 7 years)							X					X		X		X		X		X^{u}	($X^{s,r}$)
WISC-IV[t] (≥ 7 years)							X					X		X		X		X		X^{u}	($X^{s,t}$)
Substudy 2																					
Population PK[w]		X	X	X	X	X															

Definitions: Bayley-III, Bayley Scales of Infant and Toddler Development, Third Edition; ETV, Early Termination Visit; M, Month; Phe, phenylalanine; Rx, prescription; W, Week; WISC-IV, Wechsler Intelligence Scale for Children, Fourth Edition; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence, Third Edition.

[a] During Screening and Part 1, weekly visits should occur within 2 days of scheduled timepoint (ie, ± 2 days). All visits will occur at the clinic.

[b] The Part 2 assessment schedule will continue until Month 84 (7 years).

[c] Monthly visits should occur within 5 days of scheduled timepoint (ie, ± 5 days) for **M2, M3, M4, M5 and M6**. All visits will occur at the clinic.

[d] **Every subject should complete a Follow-up or Termination visit to end their participation in the study. Follow-up visit is performed for subjects who complete only Part 1 of the study, or who are enrolled into Part 2 and complete Part 2. The Termination visit is completed by subjects who withdraw or are discontinued from the**

study. Subjects will be contacted by telephone 4 weeks (28 ± 3 days) after the last dose of study drug, unless required to return for a clinic visit (see footnotes i, q, r, s, and t)."

[e] All study procedures for the W 0 visit must occur before the first dose of study drug is taken during that visit. Subjects will receive their first dose of study drug under observation after all other procedures have been performed. Physical examination, clinical laboratory tests, height and head circumference measurements will be repeated only if the W 0 visit is > 7 days after the Screening visit.

[f] Subjects will be eligible to participate in Part 2 if they have a ≥ 30% average reduction in blood Phe concentrations calculated as the mean of the weekly percent change from baseline at Weeks 1, 2, 3, and 4.

[g] 6-month visits should occur within 2 weeks of scheduled timepoint (ie, ± 2 weeks). All visits will occur at the clinic.

[h] Vital signs will be taken pre-dose and for 3 hours following the first dose in all subjects (at 15, 30, 45, 60, 90, 120, and 180 minutes post-dose).

[i] Subjects with previous laboratory test results that reveal clinically significant abnormalities will have a clinic visit at follow-up rather than a telephone visit, with repeat vital signs, physical examination, and clinical laboratory tests.

[j] Clinical laboratory tests include hematology, chemistry, and urinalysis. **Urinalysis may be performed in the clinical lab or via "dipstick" in children too young to provide a urine sample.**

[k] An ECG will be obtained 2-6 hours following the first dose of Kuvan on the first 80 subjects enrolled unless data from other studies are clear that Kuvan does not have an effect on the QT interval.

[l] Pregnancy tests will be conducted when, in the estimation of the investigator, the subject reaches child-bearing potential.

[m] Blood samples for a subject should be obtained at approximately the same time of day at each visit and will be analyzed at a local laboratory.

[n] Only serious adverse events (SAEs) are collected at the W 0 visit (after Informed Consent is signed, but prior to first dose of study drug). The reporting period for non-serious AEs begins after the first dose of study drug is administered.

[o] If a subject's blood Phe concentration drops below 120 µmol/L, **at the discretion of the investigator,** the subject's dietary Phe restriction **may** be modified to allow a **gradual** increased intake of **approximately** 5-**20** mg/kg of Phe per day**, avoiding unstable swings in the blood Phe values.**.

[p] Neurocognitive testing will be performed within 6 weeks after a subject is determined to be Kuvan responsive.

[q] Bayley-III, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects and at 6 months intervals thereafter until the subject is ≥ 30 months old. At that time, the subject will be assessed using the WPPSI-III test. If a subject leaves the study between 4 to 6 months after the preceding Bayley-III assessment, this test, or the WPPSI-III test if the subject is ≥30 months at dropout, will be done at follow-up.

[r] WPPSI-III, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects, then yearly thereafter until the subject is ≥7 **years old**. At that time, the subject will be assessed using the WISC-IV test. If a subject leaves the study between 9 months and 1 year after the preceding WPPSI-III assessment, this, or the WISC-IV test if the subject is older than 7 years at dropout, will be done at follow-up.

[s] **The WPPSI-III has two forms. The younger children will be administered the first form for ages 30 months up to 4 years old. The second form will be used for subjects 4 years of age up to 7 years old.**

[t] WISC-IV, performed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects and **every 2 years** thereafter. If a subject leaves the study between 9 months and 1 year after the preceding WISC-VI assessment, this test will be done at follow-up.

[u] **The WPPSI-III or WISC-IV will continue to be administered on an annual or bi-annual basis, dependent on subject's age, at months, 60, 72 and 84.**

[v] **3-Month interim phone contact should occur within 1 week of scheduled timepoint (ie, ± 1 week). Phone calls will be performed between clinic visits that occur after month 6 and through Month 84. Subjects will be contacted by telephone to document the subject's weight, study drug supply, AEs, and Con Meds.**

[w] The population PK study will be performed using 80 subjects, with at least **10** subjects 0-1 years old, and **70** subjects > 1-6 years old who enroll in Part 1 of the study. See Section 12.3 for details.

3 TABLE OF CONTENTS

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
ALAG	Absorption lag-time
ALT	Alanine transaminase
AST	Aspartate transaminase
Bayley-III®	Bayley Scales of Infant and Toddler Development®-Third Edition
BH4	Tetrahydrobiopterin; Kuvan formulation contains 6R-BH4 (sapropterin)
BUN	Blood urea nitrogen
CD	Compact disk
CFR	Code of Federal Regulations
CI	Confidence interval
CL/F	Apparent clearance
CRA	Clinical Research Associate
CRF	Case Report Form
d/L	Deciliter per liter
DBP	Diastolic blood pressure
DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	Electrocardiogram
ETV	Early termination visit
FDA	Food and Drug Administration
F/U	Follow-up
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act
HPA	Hyperphenylalaninemia
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IP	Investigational Product (Study Drug)

IQ	Intelligence quotient
IRB	Institutional Review Board
IV	Intravenous
Ka	Absorption rate constant
MedDRA	Medical Dictionary for Regulatory Activities
MRC	British Medical Research Counsel
NOAEL	No observable adverse effect level
PDE5	**Phosphodiesterase type 5 inhibitor**
Phe	Phenylalanine
PI	Principal Investigator
PK	Pharmacokinetic
PKU	Phenylketonuria
Q/F	Apparent inter-compartmental clearance
®	Registered trademark
REB	Research Ethics Board
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SD	Standard deviation
SDV	Source data verified
SE	Standard error
US	United States
Vc/F	Apparent volume of distribution of the central compartment
WPPSI™-III	Wechsler Preschool and Primary Scale of Intelligence™-Third Edition
WISC®-IV	Wechsler Intelligence Scale for Children®-Fourth Edition

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), Ethics Committee (EC), or Research Ethics Board (REB) is properly constituted and compliant with International Conference on Harmonisation (ICH) and Good Clinical Practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/EC will be provided to BioMarin Pharmaceutical Inc. (BioMarin) or its designee. The Principal Investigator (PI) will provide the IRB/EC with all appropriate material, including the protocol, Investigator's Brochure (IB) or Package Insert, 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/EC 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/EC or REB 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:

- US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
- E6 International Conference on Harmonisation [ICH] Guideline for Good Clinical Practice
- 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/EC; 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/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/EC. BioMarin and the IRB/EC 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 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 Kuvan® is contained in the IB or package insert supplied by BioMarin; the Investigator should review these documents prior to initiating this study.

7.1 Nonclinical Studies

Nonclinical studies were conducted in mice, rats, dogs, and marmosets at doses ranging from 20 to 4000 mg/kg and using intravenous (IV) and oral routes of sapropterin administration. In a 52-week marmoset toxicology study in which animals received oral dosages of 20, 80, or 320 mg/kg/day sapropterin, the no observable adverse effect level (NOAEL) was determined to be 320 mg/kg/day. The only observed AEs in this study were salivation and vomiting, both possibly caused by the dosing method and low pH of the dosing solution; no laboratory or histopathologic abnormalities were found. In a similar 52-week rat toxicology study, animals received oral dosages of 4, 40, or 400 mg/kg/day sapropterin. The NOAEL was determined to be 40 mg/kg/day, with a low incidence of mild increases in basophilic infiltrates in the renal collecting tubules of the animals treated with 400 mg/kg/day for 52 weeks. The renal histopathologic findings were not associated with changes in clinical chemistry or urinalysis results, and the findings were believed to be of no clinical consequence. Additionally, no renal tubular changes were found in the subsequent rat carcinogenicity study, after 104 weeks of daily sapropterin administration at doses up to 250 mg/kg/day.

Carcinogenicity studies using doses up to 2 times the maximum recommended human dose of 20 mg/kg/day were conducted in F-344 rats (2-year study) and CD-1 mice (78-week study) (Package Insert). There was an increase in the incidence of benign adrenal pheochromocytoma in rats treated at the highest dose; no carcinogenic effect was seen in the mice. At 3 times the maximum human dose of 20 mg/kg/day, no effect on fertility or reproduction function was observed in male and female rats.

These data, in addition to the data reported in the remainder of the nonclinical pharmacology, pharmacokinetic, and toxicology studies, support the safety of Kuvan.

For more information on nonclinical studies, see the IB or the Package Insert.

7.2 Previous Clinical Studies

The efficacy and safety of Kuvan were evaluated in 4 multicenter clinical studies in subjects with PKU. Study 1 was an open-label study evaluating Kuvan at 10 mg/kg /day in subjects whose blood phenylalanine (Phe) concentrations were not in control and who were not following a Phe-restricted diet. Study 2 was a double-blind, placebo-controlled study using a Kuvan dose of 10 mg/kg/day; Study 3 was a dose-titration study evaluating Kuvan dosages of 5, 10, and 20 mg/kg/day in subjects who completed Study 2; and Study 4 was an open-label study using Kuvan at a dose of 20 mg/kg/day in children ages 4 to 12 years who were on Phe-restricted diets and whose blood Phe concentrations were ≤ 480 µmol/L at screening.

All studies showed that Kuvan lowered blood Phe concentrations in a subset of subjects enrolled in each study. In the 6-week double-blind study (Study 2), the Kuvan-treated group had a mean change in blood Phe concentration from baseline to Week 6 of -239 µmol/L while the placebo-treated group mean change was 6 µmol/L; the mean percent changes (± standard deviation, SD) were -29% (± 32) (Kuvan) and 3% (± 33) (placebo). The difference between the groups was statistically significant ($p < 0.001$).

7.3 Study Rationale

Numerous investigations have revealed the devastating neuropsychological impact of elevated blood Phe concentration in adults and children with PKU (Moyle, 2007, Neuropsychol.Rev.). Rigorous control of diet is typically advocated in children 4 years and under with PKU because brain sensitivity to high Phe concentrations is expected to be greatest during these years of rapid neurocognitive development.

The strongest determinant of intelligence quotient (IQ) and cognitive function is compliance with blood Phe control. Several clinical studies with Kuvan have already demonstrated efficacy in reducing blood Phe in subjects older than 4 years. This study will examine whether addition of Kuvan to the standard of care at an early age in children with well-controlled diets can lower blood Phe levels (ie, reach and maintain a goal of ≤ 240 µmol/L) and preserve neurocognitive functioning. In addition, Part 1 of this study will provide data on Kuvan exposure, rate of uptake, half-life, and clearance in young children.

7.4 Summary of Overall Risks and Benefits

7.4.1 Summary of Risks

Response to Kuvan treatment in PKU subjects is variable. Not all subjects responded to treatment with Kuvan in clinical trials, and the initiation of Kuvan treatment does not eliminate the need to monitor for adequate blood Phe control. Prolonged elevations in blood Phe concentrations can result in neurologic impairment. Conversely, some subjects in clinical trials who were following Phe-restricted diets and received treatment with Kuvan experienced substantial reductions of blood Phe. Concentrations of blood Phe that are too low may be associated with catabolism and protein breakdown. Therefore, when Kuvan is used in combination with a Phe-restricted diet, patients should be monitored closely to ensure that blood Phe concentrations are not too low, and, if necessary, the dose of Kuvan should be adjusted or dietary Phe should be increased.

Adverse reactions have usually been mild and transient. The integrated safety database consists of 579 patients in Kuvan clinical trials. These patients were treated at daily doses of 5 mg/kg, 10 mg/kg, or 20 mg/kg, from 8 days to over 6 months. No dose-related or extent of exposure-related change in frequency of AEs was observed. Six serious adverse events occurred in clinical trials, none were considered to be study drug related. One subject withdrew from a clinical trial due to an AE of pregnancy. The most frequent AEs thought to be related to Kuvan in placebo-controlled trials were pharyngolaryngeal pain, and rhinorrhoea. The most frequently reported adverse reactions in all clinical trials (> 4%), regardless of relationship to study drug, were headache, diarrhea, abdominal pain, upper respiratory tract infection, pharyngolaryngeal pain, vomiting, and nausea. Mild to moderate neutropenia was noted during Kuvan administration in 24 of 579 subjects (4%) and in 3 of 59 subjects receiving placebo (5%).

Potential risks will be assessed in PKU-015 by recording of medical history, AEs, and vital signs; performing physical examinations and ECGs, if required; and conducting clinical laboratory tests (chemistry, hematology, and urinalysis).

7.4.2 Summary of Benefits

Prolonged high blood Phe concentrations are neurotoxic and lead to impairment of intelligence and other brain functions (such as attentiveness). Reduction of blood Phe concentrations through dietary control is an important determinant of long-term neurologic outcome in PKU patients, and reduction of blood Phe concentrations in patients with PKU has been shown to decrease the long-term risk of neurologic injury. It is difficult for many patients to maintain reduced blood Phe, and many patients with PKU experience some degree of neurological impairment despite efforts to maintain dietary Phe control.

In clinical trials with Kuvan in subjects with PKU, reductions in blood Phe concentrations were observed in some subjects. Although long-term assessment of neurologic function in subjects with PKU receiving Kuvan for the treatment of elevated blood Phe has not been assessed, Kuvan may help maintain reduced blood Phe concentrations as an adjunct to a Phe-controlled diet.

Some subjects may not benefit from participation in PKU-015; however, their participation in this study might help in the future treatment of other individuals with PKU.

8 STUDY OBJECTIVES

The primary objective of the study is:

- To evaluate the long-term efficacy of Kuvan in preserving neurocognitive function in children with PKU when treatment is initiated at 0-6 years

The secondary objective(s) of the study is (are):

- To evaluate the long-term safety of Kuvan in the study population
- To evaluate the effect of Kuvan on growth parameters in the study population

Substudy 1: 6-Month Safety and Efficacy in Children 0-6 Years Old

Primary Objective

- To evaluate the safety of 6 months of treatment with Kuvan in children with PKU who are 0-6 years old

Secondary Objective

- To evaluate the efficacy of 6 months of treatment with Kuvan in controlling blood Phe concentration within acceptable ranges in children with PKU who are 0-6 years old
- To provide baseline neurocognitive data for all Kuvan-responsive subjects and 6-month Bayley-III data for subjects who are 0-2 years old at enrollment

Substudy 2: Population Pharmacokinetics in Children 0-6 Years Old

Primary Objective

- To evaluate the population pharmacokinetics of Kuvan in young children

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

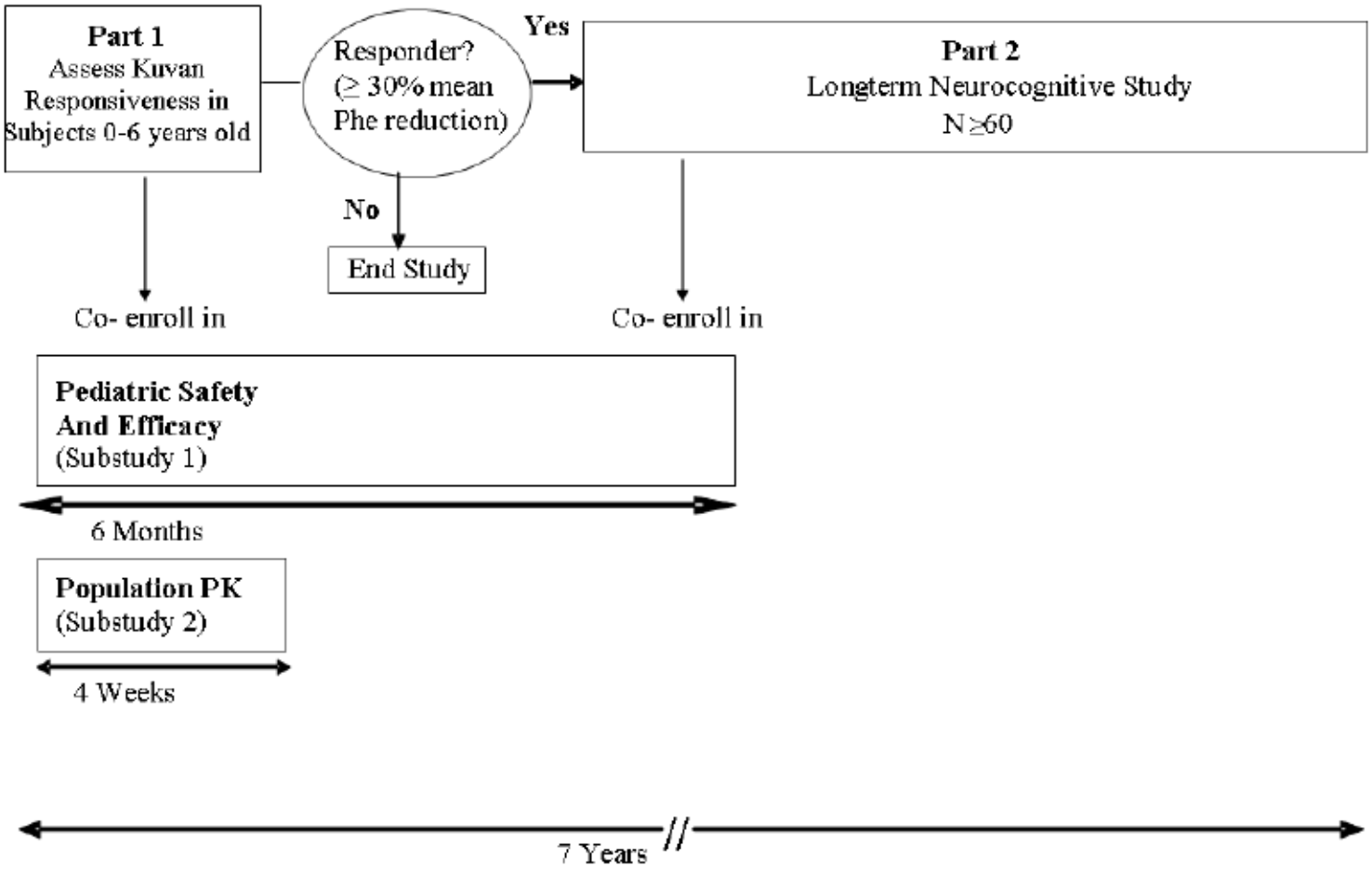
This 2-part multicenter, open-label study is designed to evaluate the safety of Kuvan and its effect on neurocognitive function, blood Phe concentration, and growth in children with PKU who are 0-6 years old. The study will be considered complete when approximately 45 subjects have completed 7 years of treatment.

One of the goals of the study will be to decrease blood Phe levels to reach a goal of ≤ 240 µmol/L. Subjects will meet with a dietician at each study visit to review dietary Phe intake. Dietary Phe may be added to the diet, at the discretion of the Investigator, if a blood Phe level falls below 120 µmol/L. See Section 9.6 for details on dietary Phe adjustment.

A safety evaluation will be conducted in these subjects during the first 6 months of the study (Substudy 1) and may include ECG testing 2-6 hours following the first dose of Kuvan. A population pharmacokinetic study will be conducted in a subset of these children during Weeks 0-4 (Substudy 2).

Figure 9.1.1 shows a schematic for the study.

Figure 9.1.1: Study Schematic



Part 1: Evaluation of Kuvan Responsiveness

Beginning at Week 0 and after all baseline assessments are completed, subjects enrolled into the study will receive 20 mg/kg Kuvan daily for 4 weeks. Subjects will return to the clinic weekly for safety assessments and blood Phe concentration measurement (Substudy 2, Population PK). Subjects should continue with their current level of dietary Phe intake. However, if a subject's blood Phe concentration drops below 120 µmol/L, **at the discretion of the Investigator, a gradual increase of approximately 5 to 20 mg/kg of** daily dietary Phe **supplement** may be **added to the diet. It is important to avoid unstable swings in the blood Phe values**. No dose adjustments can be made during Part 1 without consultation with the Medical Monitor.

Part 2: Neurocognitive Study

Subjects who are responsive to Kuvan (see the definition of responsiveness in **Inclusion and Exclusion** Criterion) will continue to receive daily single doses of 20 mg/kg Kuvan orally. At the discretion of the Investigator, the subject's daily Phe intake may be modified if blood Phe levels fall below 120 µmol/L during the study. Kuvan dose may also be reduced after Week 5 after consultation with the Medical Monitor, in subjects who do not tolerate the 20 mg/kg/day dose. Neurocognitive testing will be performed within 6 weeks of determination of Kuvan responsiveness in all Kuvan-responsive subjects. The types of neurocognitive tests used (ie, Bayley Scale of Infant and Toddler Development®-Third Edition [Bayley-III], Wechsler Preschool and Primary Scale of Intelligence™-Third Edition [WPPSI-III], and/or Wechsler Intelligence Scale for Children®-Fourth Edition [WISC-IV]), will be those considered appropriate for the subject's age.

Table 9.1.1: Neurocognitive Assessments

Age Group	Infancy	Preschool		School-Age
Assessment	Bayley-III	WPPSI-III	WPPSI-III	WISC-IV
Age	0 months through <30 months	≥ 30 months through <4 years	≥ 4 years through <7 years	≥ 7 years and older
Frequency	Every 6 months ±2 weeks	Once a year ±2 weeks	Once a year ±2 weeks	Every 2 years±2 weeks

The Bayley-III will be administered to subjects who are from 0 months to less than 30 months of age and will be administered every 6 months ± 2 weeks. At 30 months old or older, the subjects will be administered the WPPSI-III. The WPPSI-III has two forms. The younger children will be administered the first form for ages 30 months to less than 4 years old. The second form will be used for subjects 4 years of age to less than 7 years old. The WPPSI-III will be administered yearly ± 2 weeks. Beginning at 7 years old study, subjects will be administered the WISC-IV every 2 years ± 2 weeks.

Growth, blood Phe concentration, and safety will be monitored. These **and the neurocognitive** assessments will be performed every 6 to 24 months, depending on the type of assessment, until the end of the study. Refer to Table 2.1 for details.

The study will be considered complete when approximately 45 subjects have been followed for 7 years and have at least 2 post-treatment WPPSI-III and/or WISC-IV tests administered 2 years apart.

After study completion, subjects will be given the opportunity to participate in a registry study that will follow longer term outcomes.

Substudy 1: Safety and Efficacy in Subjects 0-6 Years Old

All Kuvan-responsive subjects will be enrolled in Substudy 1 and will be assessed monthly for the first 6 months of the study. Blood Phe concentrations, various measures of growth, and safety data will be collected. The subject's daily Phe intake will be increased only if the subject's blood Phe levels fall below 120 µmol/L. Kuvan dose may also be reduced **after Week 5 and** after consultation with the Medical Monitor if the subject does not tolerate the approximately 5-20 mg/kg/day dose. Refer to Table 2.1, Schedule of Events for the timing of assessments.

Substudy 2: Population Pharmacokinetics (PK) in Subjects 0-6 Years Old

For the population PK evaluation, the first **10** subjects in the 0-1 year age group and the first **70** subjects in the > 1-6 year age group will be enrolled. Three (3) plasma samples from each subject in the 0-1 year age group and 4 plasma samples from each subject in the > 1-6 year age group will be collected at the Week 0, Week 1, Week 2, Week 3, and/or Week 4 visits according to a predetermined schedule. See Section 12.3 and Table 12.3.1 for sampling windows.

9.2 Discussion of Study Design, Including Choice of Control Group

The design of this clinical study is intended to supply data to complement the results of 2 completed double-blind placebo-controlled studies that established the safety and efficacy of Kuvan in PKU-affected individuals. PKU-003 and PKU-006 demonstrated acceptable safety profiles and statistically and clinically meaningful reductions in blood Phe concentration in subjects > 8 years old and 4-12 years old, respectively. The latter study also showed statistically and clinically meaningful increased tolerance of additional Phe in the diet while overall blood Phe control was maintained. Because neither study included subjects less than 4 years old nor assessed the impact of the drug on neurocognitive development, these factors became the basis for undertaking the present study.

PKU-015 will allow assessment of safety, efficacy, and PK in a sufficient number of young subjects to provide a comparable experience with the treatment of older affected individuals in completed Kuvan clinical studies. PKU-015 comprises 0-6 year old children because this is the age range where brain tissue is particularly sensitive to the effects of elevated blood Phe. The design does not include a control group for 3 reasons. First, from the perspective of Phe reduction and overall safety, other formulations of BH4 have been used successfully across all age groups (summarized in Section 9.4.6), and there is no evidence that subjects less than 4 years old will respond to this formulation of BH4 any differently than those older than 4 years. Second, use of a placebo is not practical because Kuvan is commercially available. Third, inclusion of subjects 4-6 years old allows for direct comparisons with same-age subjects in the drug-treated and placebo arms of PKU-006.

Part 1 will allow for selection of a suitable population to carry out long-term neurocognitive assessments in Part 2. For inclusion in Part 2, a minimal level of preserved neurocognitive function documented by test scores and robust, consistent reductions in blood Phe over 4 weeks of treatment in Part 1 will ensure that the long-term benefits of Kuvan can be adequately evaluated. There is no value in enrolling subjects with irreversible deficits in IQ or those who have a history of dietary noncompliance in this study. The 3 neurocognitive tests used to cover the age range of PKU-015 subjects represent the best available tools (reviewed in Section 11.1).

A population PK study (Substudy 2) will be conducted on a subset of the subjects enrolled in Part 1. Blood will be drawn for total biopterin measurement at the Week 0, 1, 2, 3, and/or 4 visit according to the schedule described in Section 12.3.

In Part 2, neurocognitive testing will be performed in these Kuvan-responsive subjects over a 7-year period, using age-appropriate tests. During Substudy 1 safety and efficacy

assessments will be conducted weekly during Week 0 to Week 4, then monthly for Months 2 through Month 6.

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:

- Parent(s) or guardian(s) willing and able to provide written, signed informed consent after the nature of the study has been explained, and prior to any research-related procedures.
- Parent(s) or guardian(s) willing and able to comply with all study procedures.
- Female subjects of childbearing potential (as determined by the investigator) and sexually mature male subjects willing to use a medically accepted method of contraception throughout the study. Female subjects of childbearing potential willing to undergo periodic pregnancy tests during the course of the study
- Established diagnosis of PKU with hyperphenylalaninemia (HPA) documented in the medical record by at least 2 blood Phe concentrations ≥ 360 µmol/L (6 mg/dL) taken at least 3 days apart
- Documented blood Phe control (defined by the standard used at each treatment center) prior to study enrollment, if applicable (eg, the subject is old enough for these data to be collected); blood Phe concentrations for subjects < 6 months old at Screening must be considered controlled and stable by the Investigator
- Willing to adhere to a prescribed Phe-restricted diet in order to maintain blood Phe concentrations within the recommended ranges established at the subject's study site
- Age 0 to 6 years, inclusive, at Screening

Individuals eligible to participate in Part 2: Neurocognitive Study and Substudy 1 must meet **all** of the following criteria:

- Completion of Week 4 visit in Part 1
- Responsive to Kuvan during Part 1, defined as a ≥ 30% average reduction in blood Phe concentration calculated as the mean of the weekly percent change from baseline in blood Phe concentration at Weeks 1, 2, 3, and 4
- Bayley-III or IQ test score ≥ 80 when tested within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects

9.3.2 Exclusion Criteria

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

- Has known hypersensitivity to Kuvan or its excipients
- Use of Kuvan or any investigational product or investigational medical device within 30 days prior to screening, or requirement for any investigational agent prior to completion of all scheduled study assessments
- Concurrent disease or condition that would interfere with study participation or safety (eg, seizure disorder, oral steroid-dependent asthma or other condition requiring oral or parenteral corticosteroid administration, or insulin-dependent diabetes)
- **Use of phosphodiesterase type 5 inhibitor, often shortened to PDE5 inhibitor (eg.sildenafil citrate, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil)**
- 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
- Established diagnosis of primary tetrahydrobiopterin (BH4) deficiency
- History of organ transplantation
- Perceived to be unreliable or unavailable for study participation or to have parents or legal guardians who are perceived to be unreliable or unavailable
- Use of methotrexate or other medications that inhibit folate metabolism
- Serious neuropsychiatric illness (eg, major depression) not currently under medical control

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 termination visit should be carried out.

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 1 month, 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/EC. It is the Investigator's (or designee's) responsibility to obtain written permission to use protected health information per country-specific regulations, such as Health Insurance Portability and Accountability Act (HIPAA) in the US, 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 will be assigned a unique subject identifier. This unique identifier will be on all CRF pages. In legal jurisdictions where use of initials is not permitted, a substitute identifier will be used. Subjects who withdraw early from 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 Kuvan sufficient for the completion of the study.

In Part 1, beginning at Week 0, subjects will receive Kuvan at a dose of 20 mg/kg/day orally, provided in tablets that each contain 100 mg of sapropterin, for a total of 4 weeks. **For ease in administration and for ease of instruction to families, the dose can also be rounded up or down:**

For children who are older and can take their medication from a cup or glass, rounding of the dose to the nearest whole tablet is acceptable. For example, a child weighing 11 kg would have a dose of 220 mg of Kuvan that may be rounded down to 200 mg (2 tablets). A child weighing 14 kg would have a dose of 280 mg that may be rounded up to 300 mg (3 tablets).

Whether the dose is determined using Table 9.4.4.1 or by rounding the dosage up or down, be consistent with the method. This is especially important through Week 4 when responsiveness is being determined.

The first dose in Part 1 will be taken at the Week 0 visit under observation, after the completion of all other study procedures. The remaining doses in Part 1 will be taken daily with food to increase absorption, preferably at the same time each day. The Kuvan dose cannot be adjusted during Part 1 without consultation with the Medical Monitor.

Subjects who are enrolled into Part 2 will initially continue to take 20 mg/kg/day Kuvan at the same time each day with a meal. After week 5, at the discretion of the Investigator and after consultation with the Medical Monitor, a subject's Kuvan dose may be lowered if the subject does not tolerate the 20 mg/kg/day dose.

9.4.2 Identity of Investigational Product (IP)

9.4.2.1 Product Characteristics and Labeling

Kuvan (sapropterin dihydrochloride) tablets are unscored, uncoated, immediate-release tablets for oral use. Each tablet contains 100 mg of sapropterin dihydrochloride (equivalent to 76.8 mg of sapropterin base). Tablets are round, **ranging from** off-white to light yellow, **light orange/pink, beige, or** mottled **(speckled) appearance**, and debossed with "177".

9.4.3 Storage

At the study site, all study drug must be stored under the conditions specified in the Investigator's Brochure 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

Kuvan (sapropterin dihydrochloride) tablets should be administered orally with food to increase absorption, preferably at the same time each day with a meal. Kuvan tablets **can** be dissolved in 4 to 8 oz. (120 to 240 mL) of water or apple juice and taken within 15 minutes of dissolution. It may take a few minutes for the tablets to dissolve. To make the tablets dissolve faster, they can be stirred or crushed. The tablets may not dissolve completely. Small pieces may be seen floating on top of the water or apple juice. This is normal and safe for subjects to swallow. If after drinking the medicine, pieces of the tablet are still seen, more water or apple juice can be added to make sure that all of the medicine is taken. **Administration of crushed Kuvan tablets may also be stirred in soft foods such as apple sauce or lemon pudding.** A missed dose should be taken as soon as possible, but 2 doses should not be taken on the same day. If a subject vomits the dose within 30 minutes of administration, the dose should be repeated. If the dose is vomited after 30 minutes, the dose should not be repeated.

Study drug will be dispensed only by study staff qualified to perform that function under applicable local laws and regulations for the study site, according to the protocol and Schedule of Events (Table 2.1). Instructions must be given on how many tablets to take each day, how to prepare the dosing solution, and when dosing should occur.

At the discretion of the Investigator, tablets may be dissolved in as little as 5 mL water or apple juice **per tablet**, based on the subject's size and capability. For infants weighing < 5 kg, a single Kuvan tablet may be dissolved in **5** mL water or apple juice and a portion of this solution corresponding to a 20 mg/kg dose may be administered orally via an oral dosing syringe; for example, an infant weighing 3.5 kg would be administered 3.5 mL (or 70 mg) of this Kuvan solution. A dosing table for subjects weighing **10** kg and less is shown in Table 9.4.4.1.

Table 9.4.4.1: Kuvan Dosing Table for Young Children

Child's Weight (kg)	Dose Given (Milligrams)	mL of Solution	No. of 100 mg Tablets Dissolved	mL Administered to Subject
1	20	5	1	1
2	40	5	1	2
3	60	5	1	3
4	80	5	1	4
5	100	5	1	5
6	120	10	2	6
7	140	10	2	7
8	160	10	2	8
9	180	10	2	9
10	200	10	2	10

9.4.5 Method of Assigning Subjects to Treatment Groups

Not applicable

9.4.6 Selection of Doses Used in the Study

The 20 mg/kg/day dosage of Kuvan was selected for this study in order to achieve enhanced blood Phe control. In studies using different formulations of BH4 including Kuvan, average blood Phe reduction in responders at the 10 mg/kg dose has been 50%, while the average reduction in responders at the 20 mg/kg dose has been 64%. Because persistent increases as low as 100 µmol/L blood Phe during the critical early childhood period can result in adverse neurological outcomes, there is a need for tighter blood Phe control in young PKU patients.

The data described below support the safety of the 20 mg/kg/day dosage of Kuvan (6R BH4):

- Single oral 6R-BH4 doses of 7.5-20 mg/kg/day have been administered as loading doses to exclude BH4 deficiency in more than 1900 PKU patients (Bernegger, 2002, Mol.Genet.Metab) (Shintaku H, 2003, J Inherit Metab Dis).
- In published studies of long-term BH4 therapy in patients with PKU, patients received treatment for periods of 5 days to 56 months. In these studies the BH4 doses ranged from 1.7 to 20 mg/kg/day. No AEs associated with BH4 therapy were reported in these studies. (Dudesek, 2001, Eur.J Pediatr.) (Shintaku H, 2003, J Inherit Metab Dis) (Muntau, 2002, N.Engl.J.Med.) (Hennermann, 2005, Mol.Genet.Metab) (Lambruschini, 2005, Mol.Genet.Metab).
- The cumulative data from the treatment experience in multiple reports offers supplementary evidence of an adequate long-term safety profile. (Trefz, 2001, Eur.J.Pediatr.) (Blau, 2003, Eur.J.Pediatr.) (Steinfeld, 2002, Eur.J.Pediatr.) (Cerone, 2004, Mol.Genet.Metab) (Spaapen, 2003, Mol.Genet.Metab) (Lindner, 2003, Hum.Mutat.) (Lassker, 2002, J.Inherit.Metab Dis.) (Koch, 2002, Mol.Genet.Metab) (Weglage, 2002, J.Inherit.Metab Dis.).

In summary, the available data indicate that Kuvan may serve as a safe and well tolerated therapy when given at doses of 20 mg/kg/day to subjects with PKU.

9.4.6.1 Selection of Timing of Dose for Each Subject

Results from a population PK study showed that the mean half-life in the terminal elimination phase is approximately 6.69 hours (range: 3.91 to 16.6 hours). Given that it takes approximately 4 half-lives to achieve clearance, coverage would be estimated to last 26.8 hours, on average, following dosing, which supports the use of once-daily dosing. Results from stochastic simulations based on this model show that there is little accumulation with daily doses.

Taking the daily dose **at the same time of day with a meal** may help to facilitate treatment compliance.

9.4.7 Blinding

Not applicable in this open-label one-arm study.

9.4.8 Prior and Concomitant Medications

All prescription and over-the-counter 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.

9.4.9 Treatment Compliance

Subjects will be instructed to return all used and unused study drug containers at each study visit. Subject compliance with the dosing regimen will be assessed by reconciliation of the used and unused study drug. The quantity dispensed, returned, used, lost, etc. must be recorded on the dispensing log provided for the study.

9.5 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP(s) 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 PI's 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 must be willing to adhere to a prescribed Phe-restricted diet in order to maintain blood Phe concentrations within the recommended ranges established at the subject's study site. The goal for blood Phe concentration during the study is ≤ 240 µmol/L. Following standard of care, all subjects and their parent(s) or guardian(s) will meet with a study **dietician** at each visit to review dietary Phe intake. A 3-day dietary record will be kept prior

to clinic visits and reviewed at the clinic. The dietary record will be maintained with the study source documents. Dietary Phe intake will be assessed along with total caloric intake (kcals) and protein intake by the dietitian to ensure that the subject is meeting daily prescribed amounts. Adjustments to diet and/or medical food will be made per clinic guidelines to maintain sufficient intake to promote growth and development.

If blood Phe levels fall below 120 µmol/L, dietary Phe may be added to the diet. In order to determine the appropriate amount of daily Phe to be given, predefined increases in Phe supplement (infant formula, non-fat dry milk, liquid milk, dried egg white powder) will be prescribed and blood Phe levels will be monitored closely. Increases in Phe supplement will be based on **blood Phe level, age, and** ideal body weight for a subject's gender, weight, and height.

If a subject's blood Phe falls below 120 µmol/L, at the discretion of the investigator, a gradual increase of approximately 5-20 mg/kg of Phe supplement may be added to the diet. It is important to avoid unstable swings in the blood Phe values

If a subject's blood Phe level goes above 240 µmol/L, dietary Phe may be decreased by approximately 5 to 20 mg/kg or defined by the standard used at each treatment center, at the discretion of the investigator.

Once a subject reaches adequate Phe supplementation, defined as 4 stable blood Phe values (study visits or non-study visits) without any diet modifications, then natural protein foods rather than Phe-containing supplements may be prescribed. The amount of milligrams of Phe in natural foods per day to be given to the subject will be equivalent to the increase in milligrams of Phe supplement tolerated by the subject.

Conversion of Phe from the supplement to natural foods should not be done during the first 6 months of the study.

9.7 Efficacy and Safety Variables

9.7.1 Efficacy and Safety Measurements Assessed

The Schedule of Events in the Synopsis (Table 2.1) describes 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	9.7.4.1
Pharmacokinetic variables	Central laboratory	9.7.6, 12.3
Blood Phe concentrations	Local laboratory	9.7.1.4

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 had.

9.7.1.2 Physical Examination Findings

Complete physical examinations will include the evaluation of all major body systems, height, weight, and head circumference. Other body systems may be examined. **Week 1** of Part 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 mm Hg, heart rate in beats per minute, respiration rate in breaths per minute, and temperature. All subjects will be monitored at the clinic for 3 hours after the first dose of Kuvan, with vital signs taken pre-dose, and 15, 30, 45, 60, 90, 120, and 180 minutes post-dose.

9.7.1.4 Blood Phe Concentration

Samples for blood Phe concentration should be drawn simultaneously with samples drawn for PK on days when both types of blood draw are required. With the exception of **the PK samples** (Part 1), blood for Phe concentration measurements will be drawn at timepoints indicated in the Schedule of Events (Table 2.1). Local laboratories should use the same methodology throughout the study, if possible, for performing blood Phe testing.

9.7.1.5 Pregnancy Testing

Female subjects who are, in the estimation of the Investigator, of childbearing potential will have a urine pregnancy test at the timepoints specified in the Schedule of Events (Table 2.1).

Additional urine 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.

9.7.2 Primary Efficacy Variable

Neurocognitive testing will be performed within 6 weeks of determination of Kuvan responsiveness in all Kuvan-responsive subjects. Subsequent neurocognitive measurements will be obtained every 6 months until the subject is ≥ 30 months old, every 12 months until the subject reaches age 7, then every 2 years through the end of the study. The types of neurocognitive tests used for the primary efficacy variable (ie, WPPSI-III and/or WISC-IV), will be those considered appropriate for the subject's age.

9.7.3 Secondary Efficacy Variables

The first secondary efficacy endpoint is change in developmental growth factors, as measured by height, and head circumference from baseline. These measurements will be made at Screening, Week 0, Week 4, Month 3, Month 6, and every 6 months thereafter, **while the weight will be measured at every visit.** The second secondary efficacy variable is the baseline and 6-month follow up neurocognitive testing results using Bayley-III in children age 0 **to less than** 30 months old.

9.7.4 Safety Variables

9.7.4.1 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 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, Schedule of Events.
The clinical laboratory tests that will be performed in this study are shown in Table 9.7.4.1.1.

Table 9.7.4.1.1: Clinical Laboratory Tests

Blood Chemistry	Hematology	Urine Tests	Other
Albumin	Hemoglobin	Urinalysis	Blood phenylalanine
Alkaline Phosphatase	Hematocrit	Appearance	
ALT (SGPT)	WBC count	Color	Blood tyrosine
AST (SGOT)	RBC count	pH	Blood tryptophan
Total bilirubin	Platelet count	Specific gravity	
Direct bilirubin	Differential cell count	Ketones	Pharmacokinetics (total biopterin, analyzed by central laboratory
BUN	Sedimentation rate	Protein	
Creatinine		Glucose	
GGT		Bilirubin	
Total protein		Nitrite	
Calcium		Urobilinogen	
Sodium		Hemoglobin	
Potassium			
Glucose			
Uric acid			
Chloride			
CO_2			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GGT, gamma-glutamyltransferase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell. **Urinalysis may be performed in the clinical lab or via "dipstick" in children too young to provide a urine sample.**

9.7.4.2 Electrocardiograms

Electrocardiograms will be performed 2-6 hours after the first dose of Kuvan in the first 80 subjects enrolled unless data from other studies show clearly that Kuvan does not have an effect on the QT interval.

9.7.5 Substudy 1: 6-month Safety and Efficacy Study

Primary Efficacy Variables

The primary efficacy variable is blood Phe level. The goal is to lower blood Phe levels and maintain them at ≤ 240 µmol/L. In addition, baseline neurocognitive testing results will be reported for all subjects, and 6-month follow-up testing will be done using the Bayley-III test for subjects age 0-24 months at enrollment.

Safety Variables

The safety variables are the same as those described for the entire study in Section 9.7.4.

9.7.6 Population Pharmacokinetic Variables

The following PK parameters will be assessed: apparent clearance (CL/F), apparent volume of distribution of the peripheral compartment (Vp/F), apparent volume of distribution of the central compartment (Vc/F), and apparent inter-compartmental clearance (Q/F), absorption rate constant (Ka), absorption lag-time (ALAG), and drug half-life. Refer to Section 12.3 for additional details.

10 REPORTING ADVERSE EVENTS

10.1 Adverse Events

According to the ICH definition, an adverse event (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 4 weeks after the final dose (ie, F/U or 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 (see 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 serious adverse event (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 the 4 weeks after the final dose (ie, F/U or 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/EC will be done in compliance with the standard operating procedures and policies of the IRB/EC and with applicable regulatory requirements. Adequate documentation must be obtained by BioMarin showing that the IRB/EC 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.
105 Digital Drive
Novato, CA 94949

Phone: (415) 506-6179
Fax: (415) **532-3144**
Email: 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
Company: BioMarin Pharmaceutical Inc.
Address: 105 Digital Drive, Novato, CA 94949, USA
Phone: [redacted]
Fax:
Email:

11 APPROPRIATENESS OF MEASUREMENTS

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

11.1 Neurocognitive Assessments

Three age-appropriate scales will be used to assess the neurocognitive function of subjects enrolled in Part 2 of this study.

The Bayley-III is a tool for assessing all facets of development in infants within an age range of 12 months up to 30 months, with normative data available for infants as young as 16 days (Bayley, 2006, Psychological Corp.). (Bayley N, 2006, J of Psychoeducational Assessment). The test includes the following development scales: Adaptive Behavior, Cognitive, Language, Motor, and Social-Emotional. Three of the scales, Cognitive, Motor, Language, are administered with child interaction. The 2 remaining scales, Adaptive Behavior and Social-emotion, are conducted with parent questionnaires.

The WPPSI-III is a tool for assessing the intelligence of children ≥ 30 months through <7 years 3 months old. The WPPSI-III is administered on two forms as age appropriate. It provides composite scores that represent intellectual functioning in specified cognitive domains (Verbal IQ and Performance IQ) as well as the child's general intellectual ability (Full-scale IQ) (Wechsler D, 2002, Psychological Corp). (Sattler, 2004, Jerome S. Sattler, Inc.).

The WISC-IV assesses the intelligence of children 7 years 0 months through 16 years 11 months old. This test also provides composite scores that represent intellectual functioning in specified cognitive areas (Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index, and Processing Speed Index) in addition to a composite score reflecting the child's general intellectual ability (Full Scale IQ) (Wechsler D, 2004, Psychological Corp)

12 STUDY PROCEDURES

12.1 Neurocognitive Study

12.1.1 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.

12.1.2 Screening Visit(s)

After parent(s) or guardian(s) have signed an ICF, they will be screened for enrollment into the study. The following study activities will be performed at Screening (refer to Table 2.1, Schedule of Events):

- Medical history, including demographics
- Physical examination
- **Weight**
- Height and head circumference
- Vital signs
- Clinical laboratory tests
- Blood Phe concentration
- Blood tryptophan and tyrosine
- Concomitant medications
- Dietary review with dietician
- Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)

12.1.3 Part 1: Evaluation of Kuvan Responsiveness, 4-Week Treatment Visit(s)

12.1.3.1 Week 0 Visit

Within 28 days of completing screening assessments, the following study activities will be performed on Week 0 of Part 1. Assessments will be done predose unless otherwise specified:

- **Weight**
- Vital signs (monitored at pre-dose and for 3 hours after the first dose of Kuvan in all subjects)
- Administer study drug
- Concomitant medications
- Dietary review with dietician
- Assessment of SAEs (predose and postdose) and all AEs (postdose)
- PK sample (refer to Section 12.3)
- ECG 2-6 hours after the first dose of Kuvan in the first 80 subjects enrolled, if required
- Blood Phe concentration
- Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Physical examination, clinical laboratory tests, height and head circumference measurements will be repeated only if the W 0 visit is > 7 days after the Screening visit
- **Dispense Study Drug**

12.1.3.2 Week 1 Through Week 4 Visits (± 2 days)

- Vital signs
- **Weight**
- Blood Phe concentration
- PK samples (Weeks 1, 2, 3, and/or 4, refer to Section 12.3)
- Concomitant medications
- Dietary review with Phe modification (if necessary) with a dietician
- Assessment of AEs

- Dispense Study Drug

12.1.3.3 Weeks 1, 3, and 4 Visits Only (± 2 days)

- Clinical laboratory tests

12.1.3.4 Week 4 Visit Only (± 2 days)

- Physical examination
- Height and head circumference
- Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Blood tyrosine and tryptophan

12.1.4 Part 2: Neurocognitive Study in Kuvan-Responsive Subjects

12.1.4.1 Months 2-5 Only (± 5 days)

- Vital signs
- Weight
- Clinical laboratory tests
- Blood Phe concentration
- Concomitant medications
- Assessment of AEs
- Dietary review with Phe modification (if necessary) with a dietician
- Dispense Study Drug

12.1.4.2 Month 2 Visit Only (± 5 days)

- Administration of age-appropriate IQ test; this test must be completed within 6 weeks of determination of Kuvan responsiveness in Kuvan-responsive subjects who continue into the Neurocognitive Study

12.1.4.3 Month 3 additional to Months 2-5

- Height and head circumference
- Physical examination
- Blood tryptophan and tyrosine

12.1.4.4 Month 6 Visit Only (± 5days)

- Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- 6-month repeat testing with Bayley-III in children 0-2 years old at enrollment

12.1.4.5 Months 12, 18, 24, 30, etc. (6 month intervals ± 2 weeks)

- Vital signs
- Blood Phe concentration
- Weight
- Height and head circumference
- Physical examination
- Clinical laboratory tests
- Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential. (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- Concomitant medications
- Dietary review with Phe modification (if necessary) with a dietician
- Assessment of AEs
- Dispense Study Drug
- Neurocognitive testing (see Section 2.1, Summary of Events and Section 9, Investigational Plan)

12.1.4.6 Months 12, 24, 36, 48, 60,72 and 84 (annual intervals ± 2 weeks)

- Blood tryptophan, tyrosine

12.1.4.7 3-Month Interim Phone Contact (Months 9, 15, 21, 27, etc, ± 1 week)

- **Weight**
- **Assessment of AEs**
- **Concomitant Medications**
- **Dispense Study Drug**

12.1.5 Early Termination and Follow-up Visit(s) (within 28 ± 3 days of last dose of study drug)

Every subject should complete either a Follow-up or Termination Visit, to end their participation in the study. The Follow-up Visit is completed by a subject who completes only Part 1 of the study, or who is enrolled into Part 2 and completes Part 2.
The Termination Visit is completed by a subject who withdraws or is discontinued from the study. The Follow-up or Termination Visit should occur 28 days after the subject's final dose of drug. Every reasonable effort will be made to have the subject complete a Follow-up or Termination Visit. If the subject does not complete a Follow-up or Termination Visit in the clinic, minimally a follow-up phone call will be made.

Any AE that causes a subject to withdraw from the study or any clinically significant abnormal laboratory value or vital sign measurement identified at the Termination Visit will be followed by the Investigator, and may require a clinic visit.

Twenty-eight days after the final dose, the following study activities will be performed at the Follow-up or Early Termination Visit:

- Concomitant medications
- Assessment of AEs
- Dietary review with **dietician**
- Physical examination (if **at a clinic visit)**
- Vital signs (if **at a clinic visit)**
- **Weight**
- Height and head circumference (if **at a clinic visit)**
- Clinical laboratory tests (if **at a clinic visit)**
- Blood tryptophan and tyrosine (if **at a clinic visit)**
- Pregnancy test if, in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.) (if **at a clinic visit)**
- **Neurocognitive test using Bayley-III if the follow up or early termination visit is 4-6 months after the last assessment.**
- Neurocognitive tests using WPPSI III or WISC IV if the follow-up or early termination visit is 9 months or more after the last assessment (if **at a clinic visit)**

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

12.2 Substudy 1: 6-Month Safety and Efficacy Study

12.2.1 Screening and Weeks 0-4

See Sections 12.1.1, 12.1.2, and 12.1.3 for assessments that will be done at the screening visit and Week 0 through Week 4 (Month 1); these are identical to the main study.

12.2.2 Months 2-6 (± 5 Days)

- Blood Phe concentration
- Clinical laboratory tests
- Vital signs
- **Weight**
- Concomitant medications
- Dietary review with Phe modification (if necessary) with a dietician
- Assessment of AEs
- Dispense Study Drug

12.2.3 Months 3 and 6 Only (± 5 days)

- Height and head circumference
- Physical examination
- Blood tyrosine and tryptophan

12.2.4 Month 6 Visit Only (± 5 days)

- Pregnancy test, if in the estimation of the investigator, the subject has reached child-bearing potential (A serum pregnancy test will be performed in the event of any positive or equivocal urine pregnancy test result.)
- 6-month repeat testing with Bayley-III in children 0-2 years old at enrollment

12.3 Substudy 2: Population Pharmacokinetic Study in Subjects 0-6 Years Old

A population PK study will be completed in a total of 80 subjects. Of these 80 subjects, 10 will be ages 0 to ≤ 1 years and 70 will be > 1 to 6 years old.

From Weeks 0 to 4, a total of 3 plasma samples will be collected from subjects ages 0 to ≤ 1 year and 4 plasma samples from subjects > 1 to 6 years old.

Each of the samples will be collected at different times in relation to the subjects' morning Kuvan dose.

- Subjects aged 0 to ≤ 1 year will have a pre-dose sample taken at Weeks 1 and 2 and a post-dose sample taken at Weeks 1, 2, 3, or 4.

Subjects > 1 to 6 years old will have a pre-dose sample on Week 0 and 3 post-dose samples. The 3 post-dose samples:

- have 3 different sampling times
- may be obtained during any of the Week 2, 3, or 4 study visits
- may be obtained in any order

The study staff will coordinate the most convenient collection schedule for each individual subject and their parents or guardians.

The collection schedule is described in Table 12.3.1.

Table 12.3.1: Sampling Windows for Population PK Study

Age Group (Years)	Visit	Optimal Sampling Time (Hours, Minutes)	Post-dose Sampling Window	
			Lower Bound (Hours, Minutes)	Upper Bound (Hours, Minutes)
0 to ≤ 1	Week 1	Pre-dose	--	--
0 to ≤ 1	Week 2	Pre-dose	--	--
0 to ≤ 1	Week 1, 2, 3, or 4	4.9 hrs (294 min) post-dose	4.5 hrs (270 min)	5.5 hrs (330 min)
>1 to 6	Week 0	Pre-dose	--	--
>1 to 6	Week 2, 3, and/or 4[a]	0.22 hrs (13 min) post-dose	0.12 hrs (7 min)	1.2 hrs (72 min)
>1 to 6	Week 2, 3, and/or 4[a]	3.2 hrs (192 min) post-dose	2.6 hrs (156 min)	5.2 hrs (312 min)
>1 to 6	Week 2, 3, and/or 4[a]	7 hrs (420 min) post-dose	6 hrs (360 min)	8 hrs (480min)

¯Not applicable

[a] Subjects may have all 3 samples drawn at one visit, at 2 of the 3 visits, or have 1 sample drawn at each of the 3 visits

Subjects will take their dose at the same time of day with a meal, per site instructions, to obtain samples for the population PK analysis within the specified time windows (eg, before dosing and 0.22 to 8 hours or 7 to 480 minutes, after dosing). The exact times of dosing will be recorded. Blood samples will be collected **approximately** 2.5 to 5 hours after the meal to

obtain blood Phe concentrations **while in Phase I of this study (Week 0-Week 6). Population PK blood draws may alter the 2.5-5 hour blood Phe sampling time.**

In addition, investigators will be asked to collect a blood sample for total biopterin testing at the time of an SAE, when possible, if the SAE is considered by the investigator to be probably or possibly related to Kuvan.

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 Effect of Kuvan on Neurocognitive Function

14.1.1 Statistical and Analytical Plans

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

14.1.2 Primary Efficacy Analysis

The primary efficacy endpoint is the IQ score measured by the WPPSI-III or WISC-IV tests. Analysis of neurocognitive development using the IQ scores will be performed using a repeated measures model. The outcome variable will be the IQ score from WPPSI-III and/or WISC-IV tests. The slope of the test scores will be calculated. The treatment will be considered successful if the lower confidence limit of the mean change in test score over a 2-year period excludes a decline of greater than 5 points. All data will be included in a combined analysis, stratified by testing sequence used (WPPSI-III/WPPSI-III, WPPSI-III/WISC-IV, or WISC-IV/WISC-IV).

A nominal decline in IQ will be considered acceptable because research has shown that despite early and continuous dietary management of PKU, these patients have lower IQ scores than siblings or non-PKU controls matched for age, sex, and parent's education. Results from a prospective study sponsored by the British Medical Research Council (MRC) of IQ in 545 children with PKU showed that PKU patients attain mean IQ levels 8 points below the norms (Smith, 1989, Eur.J.Clin.Nutr.) A report from the PKU Collaborative Study showed that at 4 years of age, 36 PKU children who had received early and continuous dietary management of PKU had significantly lower (P = .02) mean [standard error, SE] IQ scores of 94.0 [17.6] than their matched non-PKU sibling controls, 99.0 [15.0] (Dobson, 1976, Pediatrics). Follow up of these same children at 8 years old confirmed the earlier findings; mean WISC Full Scale IQ score in the PKU children was 100 compared with 107 for their matched sibling controls (P = .001; (Koch, 1984, J.Inherit.Metab Dis.). In a study of 37 PKU patients between 3 and 19 years old who had received early and continuous dietary management of PKU, cognitive function was compared with that of controls matched for age, sex, and geographic location (Gassio, 2005, Dev.Med.Child Neurol.). The mean IQ (± standard deviation, SD) was significantly lower in the PKU group (100 ± 11) compared with that of the control group (111 ± 8.8) (P = .001). Similarly, (Ris, 1994, J.Pediatr.) compared 10 adults with PKU whose disease was dietary managed early in life with their unaffected control siblings. Again, PKU patients had significantly lower mean IQ results (91 ± 12) than did their non-PKU siblings (100 ± 11, P = .04). Therefore, because patients

with PKU whose blood Phe concentrations have been managed early and continuously with a Phe-restricted diet are not expected to have normal IQ, a nominal decline of up to 5 IQ points over a 2-year period will be considered the expected baseline measurement for the study.

14.1.3 Secondary Efficacy Analysis

The secondary efficacy endpoint is:

Change in developmental growth factors, measured by height, weight, and head circumference from baseline

This secondary endpoint will be analyzed using a longitudinal model.

14.1.4 Safety Analysis

The original terms reported in CRFs to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent 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 had a SAE, including death, or had an AE resulting in early discontinuation of study drug.

Laboratory data will be summarized by the type of laboratory test. The frequency and percentage of subjects who experience an abnormality (ie, outside of reference ranges) or clinically significant abnormality after study drug administration will be presented for each laboratory analyte. For each laboratory analyte, descriptive statistics will be provided for values measured at each scheduled visit, and for change in values from baseline to each scheduled visit. Similarly, descriptive statistics of vital signs will be provided.

Interim safety analyses will be conducted annually through completion of the study.

14.1.5 Determination of Sample Size

The calculation of sample size assumes that each child will have 2 post-treatment test results from the WPPSI-III and/or WISC-IV tests at least 2 years apart. The goal is to exclude a mean 2.5-point loss per year in score. Given a 2-sided Type I error rate of 0.05, if the mean IQ score at baseline is 100, the standard deviation is 15, and the correlation between the 2 test results is 0.8, a sample size of 45 subjects will yield 90% power.

To account for the possibility of dropouts and to have reasonable precision for estimating change in IQ from the time a child reaches at least age 30 months until the end of the study, approximately 60-80 Kuvan-responsive subjects will be enrolled in the study.

14.2 Substudy 1: Safety and Efficacy Analyses

Efficacy will be assessed by the ability of Kuvan, administered over a 6-month period, to control blood Phe concentrations within acceptable ranges for age and for the criteria used at the treatment centers. Efficacy endpoints are:

- Proportion of subjects whose blood Phe concentration is within acceptable ranges at post-treatment visits
- Mean change in blood Phe concentration from baseline to post-treatment monthly visits

An exact 95% confidence interval (CI) for the proportion of subjects whose blood Phe concentration within acceptable ranges at each post-treatment visit and a 95% CI for the mean change in blood Phe concentration from baseline to post-treatment monthly visits will be provided for the efficacy assessment. Baseline blood Phe concentration will be calculated using the mean of Screening and Week 0 (prior to dosing with Kuvan) results.

Safety results for the first 6 months of treatment will be analyzed as described above.

14.3 Substudy 2: Pharmacokinetic Analyses

The time course of the calculated BH4 concentration (from plasma total biopterin measurement) will be modeled to estimate the following population PK parameters for Kuvan: apparent clearance (CL/F) with associated inter-individual variability, apparent volume of distribution (Vc/F) with associated inter-individual variability, and absorption rate constant with associated inter-individual variability (Ka). Data from the Substudy 2 may also be pooled with PK data obtained from adult subjects in previous clinical studies.

14.4 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/EC must be sought, and the Investigator should inform BioMarin and the full IRB/EC within 2 working days after the emergency occurs.

With the exception of minor administrative or typographical changes, the IRB/EC 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/EC 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/EC, 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)

This open-label one-arm study will not have a DMC.

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/EC 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 developed and 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 source data verified 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, 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 U.S. 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 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/EC 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/EC. Additionally, he or she will not make any changes in the research without IRB/EC 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: A Phase 3b Open-label Study to Evaluate the Effect of Kuvan® on Neurocognitive Function, Maintenance of Blood Phenylalanine Concentrations, Safety, and Population Pharmacokinetics in Young Children with Phenylketonuria

Protocol Number: PKU-015, Amendment 2

I have read the forgoing amended 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] 1 December 2010

Medical Officer Signature [redacted] Date

Printed name: [redacted]