A Pivotal, Phase 1, Randomized, Open-Label, Single-Dose, Two-Way Crossover, Bioequivalence Study of Sapropterin Dihydrochloride 100 mg/mL Oral Suspension (Product Code: RLF-OD032) and Kuvan® (sapropterin dihydrochloride) 100 mg Powder for Oral Solution in Healthy Participants under Fed Conditions

Protocol N^{O}./Sponsor Study N^{O}: 2024-5705
Version: 1.0 (June 13, 2025)

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2.0 Synopsis

Title:	A Pivotal, Phase 1, Randomized, Open-Label, Single-Dose, Two-Way Crossover, Bioequivalence Study of Sapropterin Dihydrochloride 100 mg/mL Oral Suspension (Product Code: RLF-OD032) and Kuvan® (sapropterin dihydrochloride) 100 mg Powder for Oral Solution in Healthy Participants under Fed Conditions
Developmental Phase:	1 (Bioequivalence)
Protocol N^{O}./Sponsor Study N^{O}:	2024-5705 Version: 1.0 (June 13, 2025)
Objectives:	**Primary Objective:** To evaluate the bioequivalence (BE) between: • Sapropterin dihydrochloride 100 mg/mL oral suspension from APR Applied Pharma Research s.a., Switzerland and • Kuvan® (sapropterin dihydrochloride) 100 mg powder for oral solution from BioMarin Pharmaceutical Inc., USA following a 10 mg/kg single dose in healthy adult participants under fed conditions **Secondary Objective:** To evaluate the safety and tolerability of the study treatments
Endpoints:	**Primary Pharmacokinetic (PK) Endpoints:** The following PK parameters will be estimated using a non-compartmental approach: • Uncorrected and baseline-corrected sapropterin AUC_t, AUC_{inf}, and C_{max} **Secondary PK Endpoints:** o Uncorrected and baseline-corrected sapropterin T_{max} and T_{lag} o Uncorrected and baseline-corrected sapropterin T_{half}, Residual area, K_{el}, Cl/F, and V_z/F **Secondary Safety Endpoint:** There will be no formal statistical evaluation of safety or tolerability. An assessment of safety will be based primarily on the incidence, frequency, and severity of adverse events (AEs).

Study Design:	Open-label, single-dose, randomized, two-period, two-treatment, two-sequence, crossover, two-stage adaptive, BE study
Sample Size:	Maximum of 100 participants: • Stage 1: Forty-two (42) participants • Stage 2: If required, the total sample size re-estimation will be conducted after Stage 1. Based on the results of Stage 1, additional participants will be enrolled. Stage 2 will follow the same study procedures of Stage 1. • Both stages may be conducted in groups
Study Population:	Healthy, light-smoking (≤10 cigarettes per day) or non-smoking, male and female adults participants, from 18 to 50 years of age
Test Product (Treatment A):	Sapropterin dihydrochloride 100 mg/mL oral suspension (Product code: RLF-OD032) (Manufacturer: Groupe PARIMA Inc., Canada; Manufactured for: APR Applied Pharma Research s.a., Switzerland)
Comparator Product (Treatment B):	Kuvan® (sapropterin dihydrochloride) 100 mg powder for oral solution (BioMarin Pharmaceutical Inc., USA)
Dose:	The dose for each participant will be calculated by multiplying the participant's weight measured at Period 1 check-in by 10 mg/kg and then rounding up to the next 100 mg dose. For example, the weight of a 70.5 kg participant would be multiplied by 10 mg/kg, resulting in 705 mg, and after rounding up, this participant would be assigned a dose of 800 mg. **Treatment A:** one (1) × 10 mg/kg dose of 100 mg/mL oral suspension **Treatment B:** one (1) × 10 mg/kg dose of 100 mg powder for oral solution
Drug Administration:	**Treatment A:** Oral, single dose, without water **Treatment B:**

	Oral, single dose, dissolved in 120±2mL of water followed by two (2) rinses of the dosing cup with 60±1 mL (total of 120±2 mL) of water each time
Meals:	• Standardized high-fat, high-calorie breakfast 30 minutes prior to drug administration • Fasting for at least 4 hours post drug administration • Standardized meals provided throughout confinement
Clinic Confinement:	At least 10.5 hours pre-dose until at least 24 hours post-dose
Smoking Breaks:	No smoking breaks will be allowed during confinement
Washout:	At least seven (7) days, no more than 14 days, between drug administrations in Periods 1 and 2
Safety Monitoring:	• Temperature: daily during confinement • Vital signs (taken after participants have been resting for at least three [3] minutes in a sitting position) (blood pressure [BP] and pulse rate [PR]): pre-dose (0-hour) and at 3 and 5 hours post-dose • 12-lead electrocardiogram (ECG) (recorded after participants have been resting in a semi-recumbent or supine position): check-in (from check-in to dosing) and at 4 and 6 hours post-dose • Health monitoring: throughout the study • Adverse events: monitored throughout the study • Investigator monitoring: prior to drug administration and until four (4) hours after the last participant is dosed and on-call throughout the study
Pharmacokinetic Sampling Schedule:	Pre-dose (-1, -0.5, and 0-hour) and at 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 12, 16, 20, and 24 hours post-dose (22 time points)
Total Blood Volume:	Pharmacokinetic (PK): Approximately 175 mL Clinical laboratory tests: Approximately 30 mL
Pharmacokinetic Sample Collection, Processing, and Storage:	• Four (4) mL blood collection tubes • Samples will be protected from UV light at all times during collection and processing

	• Procedures for collection, processing, and shipping of PK blood samples will be detailed in a laboratory manual
Analytical:	Sapropterin concentrations will be measured from plasma by a validated analytical method
Statistical Analysis:	Analysis of Co-Variance (ANCOVA) will be applied for baseline-corrected analysis on log-transformed AUC_t, AUC_{inf}, and C_{max} parameters, using the actual total dose included in the ANCOVA statistical model as a covariate. The ratio of geometric least square means for treatments and the corresponding confidence interval (CI) for the ratio of geometric least square means, based on least-squares means from the ANCOVA of the log-transformed data, will be calculated for AUC_t, AUC_{inf}, and C_{max}. Uncorrected and baseline-corrected data will be presented. Additional PK statistical analysis may be performed. For example, an analysis with potency corrected data (to adjust the ratio and CI for the comparison between Test and Comparator).
Interim Analysis, Criteria for Initiation of Stage 2 and Criteria for Evaluation:	An interim analysis on AUC_t, AUC_{inf}, and C_{max}[1] will be performed after the 42 participants have completed Stage 1. Estimates of the within-subject variance for these parameters will be obtained. The power will be estimated using this variance estimate from Stage 1, assuming an α level of 0.05 and a geometric mean ratio (GMR) of 0.95. • If all power estimated from AUC_t AUC_{inf} and C_{max} ≥80%, a 90% (α=0.05) CI for the AUC_t, AUC_{inf}, and C_{max} true GMR and a point estimate will be calculated. The BE criteria will be evaluated based on the results of analysis from Stage 1 where the 90% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR has to fall completely between 80.00 and 125.00%. Regardless of the outcome of the BE evaluation, Stage 2 will not be required, and the study will be considered complete with the subjects from Stage 1 only and a conclusion supporting BE or not will be made. • If at least one power estimated from AUC_t, AUC_{inf},and C_{max} <80%, a 94.12% (α=0.0294) CI for the AUC_t, AUC_{inf}, and C_{max} true GMR will be calculated. If the results of the analysis from Stage 1 indicate that the

94.12% CI for the AUC_t, AUC_{inf}, and C_{max} true GMR falls completely between 80.00 and 125.00%, Stage 2 will not be required, and the study will be considered complete with the participants from Stage 1 only. If the results from Stage 1 indicate that the 94.12% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR does not fall completely between 80.00 and 125.00%, Stage 2 will be initiated.

- If BE is not demonstrated, the study can continue with Stage 2. The sample size for Stage 2 is to be estimated using the variance at Stage 1, GMR of 0.95, and an α level of 0.0294. The BE is evaluated at Stage 2 using data from both stages (α=0.0294) where the 94.12% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR has to fall completely between 80.00 and 125.00%.

2.1 Study Flowchart

Stage 1

Sequence	Period 1	Washout (at least 7 days)	Period 2
AB	Treatment A Test Product		Treatment B Comparator Product
BA	Treatment B Comparator Product		Treatment A Test Product

Interim Analysis: PK and statistical analysis of Stage 1 data will be conducted after the completion of Stage 1.

Stage 2

If required, Stage 2 will be conducted after Sponsor has reviewed the data from Stage 1.

Sequence	Period 1	Washout (at least 7 days)	Period 2
AB	Treatment A Test Product		Treatment B Comparator Product
BA	Treatment B Comparator Product		Treatment A Test Product

2.2 Study Procedures – Stages 1 and 2[ab]

Procedure		Screening	Period 1			Washout	Period 2			End-of-Study for Stages 1 and 2 or Early Termination[c]
			Check-in				Check-in			
Day		**-29 to 0**	**0**	**1**	**2**	**1-8**	**7**	**8**	**9**	**9**
Privacy Consent		X								
Volunteer Consent		X								
Informed Consent			X							
Medical History		X[d]								
Inclusion/Exclusion Criteria and Ongoing Eligibility Confirmation		X	X[e]				X[f]			
Demographic Data Collection		X	X							
Height and Weight Measurement with BMI calculation		X								
Weight measurement for dose calculation			X							
Physical Examination			X							X
Clinical Laboratory Tests (on urine and blood)		X								X
Serology Tests		X								
Serum β-human Chorionic Gonadotropin (β-hCG) (Females)		X	X				X			X
12-Lead ECG Recording[g]		X	X[h]	X			X[h]	X		X
Vital Signs[i]	BP and PR	X		X				X		X
	Temperature		X	X	X		X	X	X	
	Respiration Rate (RR)									
Urine Drug Screen		X	X				X			
Urine β-hCG (Females)			X				X			

[a] For each participant, the expected duration in this study will be approximately 10 days.
[b] Following completion of Stage 1, interim analysis will be conducted to determine if Stage 2 is required. Stage 2 screening will be done within 30 days prior to Stage 2 first drug administration. The duration of this study will be extended to include the total number of days required to complete Stage 1, the interim analysis, and Stage 2.
[c] End-of-study will be completed at the end of Stage 1 and Stage 2, if it is required.
[d] Medical history includes health and medication information.
[e] Any outstanding procedures/confirmation from screening and by inclusion/exclusion criteria checklist
[f] Confirmation of ongoing eligibility based on the outcome of the tests/procedures performed at Period 2 check-in.
[g] ECG will be conducted after participants are resting in a semi-recumbent or supine position.
[h] ECG at check-in will be completed in the interval from check-in to dosing.
[i] Vital signs will be taken after participants have been resting for three (3) minutes in a sitting position.

Procedure	Screening	Period 1			Washout	Period 2			End-of-Study for Stages 1 and 2 or Early Termination[c]
		Check-in				Check-in			
Day	**-29 to 0**	**0**	**1**	**2**	**1-8**	**7**	**8**	**9**	**9**
Breath Alcohol Test		X				X			
High-Fat, High-Calorie Breakfast			X				X		
Drug Administration			X				X		
Drug Accountability from Re-Packaging to Administration			X				X		
PK Blood Collection			X	X			X	X	
Interim Analysis									X[j]
Discharge from Study									X
Monitor/Record Adverse Events and Concomitant Treatments		X	X	X	X	X	X	X	X

[j] Interim analysis will be conducted only at the end of Stage 1.

2.3 Revision History

This is the initial protocol. There are no revisions currently.

3.0 List of Abbreviations

AE	Adverse Event
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Co-Variance
AST	Aspartate Aminotransferase
AUC_{inf}	The area under the analyte concentration versus time curve from time zero to infinity
AUC_t	The area under the analyte concentration versus time curve from time zero to the time of the last measurable analyte concentration (t)
β-hCG	Beta Human Chorionic Gonadotropin
BLQ	Below Limit of Quantitation
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDASH	Clinical Data Acquisition Standards Harmonization
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
Cl/F	Apparent clearance
C_{max}	Maximum measured plasma analyte concentration over the sampling period
CRF	Case Report Form
CRO	Contract Research Organization
CTA	Clinical Trial Application
CV	Coefficient of Variation
CYP	Cytochrome P450
DHHS	Department of Health and Human Services
e.g.	exempli gratia (for example)
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
ERB	Ethics Review Board
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transpeptidase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GMR	Geometric Mean Ratio
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IUD	Intrauterine Device
K_{el}	Apparent first-order elimination rate constant
LD	Lactate Dehydrogenase
LQCT	Last Quantifiable Concentration Time
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter

NTEAE	Non-Treatment Emergent Adverse Event
PCF	Privacy Consent Form
PK	Pharmacokinetic
PMRI	Pharma Medica Research Inc.
PR	Pulse Rate
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
rpm	Revolutions Per Minute
R^2	Coefficient of determination obtained from regression analysis
RR	Respiration Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
T_{half}	The apparent elimination half-life
TLIN	Start time for linear regression
T_{half}	The apparent elimination half-life
T_{lag}	Time of observation prior to the first observation with a measurable (non-zero) concentration
T_{max}	Time of the maximum measured analyte concentration over the sampling period
U.S./USA	United States of America
VCF	Volunteer Consent Form
V_z/F	Apparent volume of distribution
WBC	White Blood Cell

4.0 Table of Contents

4.1 List of Tables

5.0 Key Personnel and Facilities

Key Pharma Medica Research Inc. Staff

Principal Investigator:	Mark L. Freedman, BSc, MD, FRCPC
Protocol Writer:	Lalitha Velayutham, MD

Study Site and Facilities

Clinical Facility:	Pharma Medica Research Inc. 4770 Sheppard Avenue East Toronto, Ontario, M1S 3V6, Canada Phone: (416) 759-4111 Fax: (416) 759-2869
Clinical Laboratory:	Alpha Laboratories Incorporated 1262 Don Mills Road Toronto, Ontario, M3B 2W7, Canada Phone: (416) 449-2166 Fax: (416) 449-0816
Bioanalytical Laboratory:	Syneos Health 2500, rue Einstein Québec (Québec), Canada G1P 0A2 Tel: 418-800-2265
Pharmacokinetic and Statistical Analyses:	Pharma Medica Research Inc. 6100 Belgrave Road Mississauga, Ontario, L5R 0B7, Canada Phone: (905) 624-9115 Fax: (905) 624-4433

Key Sponsor Staff

Chief Scientific Officer: Giorgio Reiner

Table 1 Pharmacokinetic Parameters of Sapropterin Dihydrochloride 10 mg/kg Administered as Intact or Dissolved Tablets in the Fasted State, and as Intact Tablets in the Fed or Fasting States

	Dosing Regimen		
Parameter	Intact/Fasting (n = 30)	Dissolved/Fasting (n = 30)	Intact/Fed (n = 30)
C_{max}, ng/mL	84.1 (42.2)	63.0 (28.5)	105 (32.1)
T_{max}, median (range), h	2.77 (1–5)	5.00 (1–6)	4.50 (2–5)
AUC_{0-t}, ng · h/mL	474 (235)	337 (186)	635 (246)
$AUC_{0-\infty}$, ng · h/mL*	559 (220)	511 (237)	752 (307)
λ_z, h^{-1}*	0.21 (0.08)	0.18 (0.09)	0.27 (0.08)
$t_{1/2}$, h*	3.67 (1.16)	4.65 (2.03)	2.80 (1.05)

*Because λ_z could not be determined for some subjects due to insufficient concentration data in the terminal phase of the plasma curve, it was not possible to calculate $t_{1/2}$ and $AUC_{0-\infty}$ for these subjects. Thus, the number of subjects for the intact/fasting, dissolved/fasting, and intact/fed regimens in this comparison were 14, 17, and 18, respectively.

Based on the clinical pharmacology and biopharmaceutics review of Kuvan®, the PK parameters of sapropterin dihydrochloride 100 mg powder for oral solution are expected to be comparable to the 100 mg tablets based on their composition, solubility, and osmolarity.[5] The powder formulation contains the same active ingredient in the same concentration, exhibits rapid dissolution using the dissolution method for sapropterin dihydrochloride tablets, has similar osmolarity in water, and its inactive ingredients are not expected to affect drug absorption and bioavailability.[4] Absolute bioavailability or bioavailability for humans after oral administration is not known. In human plasma (*in vitro*), the protein-binding rate remained constant (22%–34%) within the concentration range of endogenous levels (approximately 3-10 ng/mL).[3]

Sapropterin is a synthetic form of BH4 and is expected to be metabolized and recycled by the same endogenous enzymes. *In vivo* endogenous BH4 is converted to quinoid dihydrobiopterin and is metabolized to dihydrobiopterin and biopterin. The enzymes dihydrofolate reductase and dihydropteridine reductase are responsible for the metabolism and recycling of BH4.[3]

The mean elimination half-life in PKU patients was approximately 6.7 hours (range 3.9-17 hours). This is comparable with values seen in healthy subjects (range 3.0-5.3 hours). There was minimal evidence to suggest drug accumulation at the highest daily dose (20 mg/kg).[3]

In-house PK data of sapropterin dihydrochloride under fed conditions are presented in the following table:

Table 2 Pharmacokinetic Characteristics of Sapropterin Dihydrochloride 100 mg Powder for Oral Solution

	T_{max} (h) Median (Range)	**C_{max} (ng/mL) Mean (±SD)**	**T_{half} (h) Mean (±SD)**
Plasma uncorrected sapropterin	4.78 (4.50 - 5.52)	127.99 (±37.39)	4.88 (±1.09)
Plasma baseline-corrected sapropterin	4.78 (4.50 - 5.52)	125.87 (±36.82)	3.38 (±1.03)

7.3 Study Rationale

APR Applied Pharma Research s.a. is developing an oral suspension of 100 mg/mL sapropterin dihydrochloride. This study will compare the bioavailability of the Test formulation to a Comparator Product (Kuvan® [sapropterin dihydrochloride] 100 mg powder for oral solution coming from the US market) under fed conditions.

Bioequivalence between the Test and Comparator Products will be determined by a statistical comparison of the AUC_t, AUC_{inf}, and C_{max} parameters for sapropterin.[6,7]

7.4 Risk-Benefit Assessment

Only healthy participants will be enrolled into this study. Participants will not gain any personal medical benefit from participating in this study.

Risks to participants in this study are related to common procedures performed (e.g., venipuncture) and the documented adverse events (AEs) listed in the current Comparator Product information. Participants are also at risk of exposure to infectious diseases, such as flu (influenza), cold (typically caused by a rhinovirus), or COVID-19, by being in the presence of people who may have a virus but do not have any symptoms. These risks are communicated to the participants in the consent forms. In case of the re-emergence of COVID-19, or any other infectious disease, safety precautions such as masking, distancing, testing, etc. may be reinstated as deemed necessary by the study doctor and/or according to local public health guidelines.

In other clinical trials, the following AEs (≥ 4% and higher than placebo) have been reported by phenylketonuria patients after taking sapropterin:[2]

- Headache (15%)
- Rhinorrhea (11%)
- Pharyngolaryngeal pain (10%)
- Diarrhea (8%)
- Vomiting (8%)
- Cough (7%)
- Nasal congestion (4%)

8.0 Drug Products

Table 3 Drug Products

	Treatment A (Test Product)	Treatment B (Comparator Product)
Drug Name	Sapropterin dihydrochloride 100 mg/mL oral suspension	Kuvan® (sapropterin dihydrochloride) 100 mg powder for oral solution
Strength	100 mg/mL	100 mg
Dosage Form	oral suspension	powder for oral solution
Route of Administration	Oral	oral
Active Ingredient	sapropterin dihydrochloride	sapropterin dihydrochloride
Manufacturer	Manufacturer: Groupe PARIMA Inc., Canada, Manufactured for: APR Applied Pharma Research s.a., Switzerland	BioMarin Pharmaceutical Inc., USA
Marketing Authorisation Holder	N/A	BioMarin Pharmaceutical Inc., USA
Dose	one (1) × 10 mg/kg dose of 100 mg/mL	one (1) × 10 mg/kg dose of 100 mg

8.1 Drug Accountability

The Sponsor will supply a sufficient quantity of the study drugs to allow for completion of this study and for sample retention. Each drug product (Test Product and Reference Product Boxes) will be numbered sequentially (e.g., 01, 02, etc.).

Designated clinical staff will be responsible for monitoring and documenting the receipt, storage, repackaging, and accounting of study drugs according to Good Clinical Practice (GCP) (ICH E6[R3]).[8]

The product name, strength, and lot/batch number will be included on each original drug container. The Comparator Product will also include an expiry date.

A randomization sequence will be created for each product to decide which package numbers will be used for the study.

8.2 Drug Receipt

Study drugs will be received by the pharmacy staff.

Data logger(s) will be downloaded by pharmacy staff and be checked thereafter. Out of range temperature will be reported to sponsor for further action taken.

Drug products will be verified and accounted against accompanying documents and protocol.

Drug products will be checked for any damage and verified for proper quantity, integrity of seal and temperature conditions.

Drug product labels will be verified to ensure they have the correct information.

Drug receipt and initial accountability procedures will be completed by delegated staff. The initial drug accountability forms will be completed for each drug product.

Drug receipt forms will be completed for each product.

An English/French Label will be generated and affixed to each bottle/ sachet in order to comply with Health Canada requirements.

8.3 Drug Storage

The study drugs will be stored in the pharmacy room within a locked cabinet with temperatures at 20-25°C (excursions permitted from 15-25°C) and protected from light.

Any out-of-range temperature will be reported to the sponsor for further action.

8.4 Drug Repackaging/Allocation and Preparation

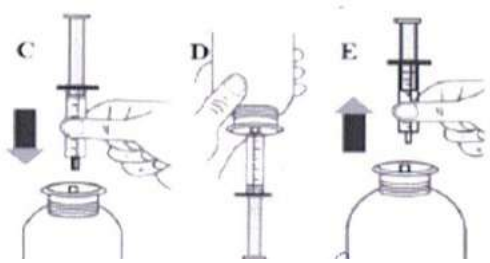
For each participant, the time of the drug preparation for both Treatment A and B, and the output of the dose calculation, will be reported on the drug repackaging form. Drug preparation will occur in the dosing area to ensure the scheduled drug administration times are met for Treatment A and Treatment B. The study drug will be prepared by pharmacy staff for each participant within 15 minutes prior to their scheduled drug administration time. Study drugs will not be prepared for participants who withdraw more than 15 minutes prior to their scheduled dosing time. If a standby participant is to be dosed, pharmacy staff will prepare the study drug within 15 minutes prior to the standby's drug administration time.

<u>Treatment A</u>

1) Participants who are assigned Treatment A according to the randomization scheme for each period will have their dose repackaged, by delegated staff, according to their weight in 5 mL syringes under UV protected light within 15 minutes prior to their scheduled drug administration time. The scheduled drug administration time can be found on each participant's eCRF.

The dose for each participant will be calculated be multiplying the participant's weight measured at Period 1 check-in by 10 mg/kg and then rounding up to the next 100 mg dose e.g., the weight of a 70.5 kg participant would be multiplied by 10 mg/kg, resulting in 705 mg and after rounding up, this participant would be assigned a dose of 800 mg (8 mL of Sapropterin dihydrochloride 100 mg/mL oral suspension). These calculations will be made by the delegated staff.

2) The following steps will be taken in each period when re-packaging Treatment A for the participant's dose:
   a) New bottle(s) will be opened in each period. Any remaining product left over in opened bottles will not be used again and will be discarded at the end of the study.
   b) All bottles will be pre-numbered and every numbered bottle that is used or allocated for a participant will be documented (e.g., S01, S03, S05 used bottle 01), even if not used for any reason.
   c) Shake the bottle vigorously for 20 seconds for each use.
   d) Open the bottle.
   e) Insert the 5 mL syringe into the Adaptor and draw out the required volume (see Table 4) from the inverted bottle (See pictures C to D).



   f) Remove the appropriately filled syringe from the bottle in the upright position (see picture E).
   g) If the participant is receiving more than one (1) syringe, the staff must ensure that each syringe is repackaged with at least three (3) mL of suspension. See below table for dose and syringes used.

Table 4 Syringe + Dose Quantity by Weight

Weight (kg)	Dosed Strength (mg)	Syringe + Dose Quantity (mL)
45.0 – 50.0	500	1 syringe with 5 mL
50.1 – 60.0	600	1 syringe with 3 mL + 1 syringe with 3 mL
60.1 – 70.0	700	1 syringe with 3 mL + 1 syringe with 4 mL
70.1 – 80.0	800	1 syringe with 4 mL + 1 syringe with 4 mL
80.1 – 90.0	900	1 syringe with 4 mL + 1 syringe with 5 mL
90.1 – 100.0	1000	1 syringe with 5 mL + 1 syringe with 5 mL

3) Study drug labels for the designated treatment will be generated and affixed to 5 mL syringes during drug repackaging with the following information: Study Number, Period, Participant Number, Treatment Designation, Product Name, Dosage Form, Strength, Quantity, Lot/Batch Number, Expiry Date, Storage Conditions, Route of Administration, Principal Investigator's Name, Pharma Medica Research Inc. Address and Telephone Number as well as a statement "For Clinical Trial Use Only" or equivalent wording. If more than 1 syringe is used, the syringes will be numbered as 'Syringe 1 of 2' and 'Syringe 2 of 2'.

4) Repackaged syringes will be placed in a covered container and protected from light until drug administration at room temperature 15-25°C.

Treatment B

Treatment B does not require drug repackaging as it is available in 10 mg pouches which are allocated to each participant, based on their weight:

1) Participants who are assigned Treatment B according to the randomization scheme for each period will have required quantity of their pouches allocated according to their weight and placed into a bag within 24-48 hours prior to drug administration. The scheduled drug administration time can be found on each participant's eCRF.

   The dose for each participant will be calculated by multiplying the participant's weight measured at Period 1 check-in by 10 mg/kg and then rounding up to the next 100 mg dose e.g., the weight of a 70.5 kg participant would be multiplied by 10 mg/kg, resulting in 705 mg and after rounding up, this participant would be assigned a dose of 800 mg (8 pouches of Kuvan® [sapropterin dihydrochloride] 100 mg powder for oral solution). These calculations will be made by the delegated staff.

2) Study drug labels for Treatment B will be generated and affixed to the bag containing all the pouches allocated to a participant during drug allocation with the following information: Study Number, Period, Participant Number, Treatment Designation, Product Name, Dosage Form, Strength, Quantity, Lot/Batch Number, Expiry Date, Storage Conditions, Route of

Administration, Principal Investigator's Name, Pharma Medica Research Inc. Address and Telephone Number as well as a statement "For Clinical Trial Use Only" or equivalent wording.

3) Prior to drug administration, the participants receiving Treatment B according to the randomization scheme will have their dose prepared under UV protected light within 15 minutes prior to their scheduled drug administration time.

4) The following steps will be taken in each period when preparing Treatment B for the participant's dose:
   - Remove the pouches from the participant's bag;
   - Tap each pouch gently to ensure all the powder settles at the bottom of the pouch;
   - Cut the pouch open (one pouch at a time):
   - Pour the powder contents of the pouch into a participant labeled cup of 120± 2 mL room temperature water;
   - Carefully stir the contents of the cup until the powder is completely dissolved; and
   - Prepared dosage cup will be placed in a tray covered and protected from light until drug administration at room temperature 15-25°C.

Further details may be provided in the Pharmacy Plan.

8.5 Drug Retention and Residual product

Residual Products

For Treatment A (Test Product), all used, empty unit-dose syringes will be returned by delegated staff to the pharmacy, inspected and discarded in each period.

Test Product bottles used for repackaging Treatment A will be accounted for and discarded at study completion after inspection.

Applicable comments will be made on the drug accountability forms prior to disposing the bottle(s) with remaining drug inside.

For Treatment B (Comparator Product), all pouches which were allocated and opened for the participant's prepared dose will be retained in their initial bags in the pharmacy after completion of drug administration in each period and discarded at study completion after inspection.

Applicable comments will be made on the drug accountability forms prior to disposing the pouch(s).

Reconciliation will be performed at the end of each period and at the end of the study. At the end of each period, a copy of the accountability forms will be sent to the Sponsor**.**

All unused bottles of Treatment A which are sealed will be counted and documented on the drug accountability form. All unused pouches of Treatment B which are sealed will be counted and documented on the drug accountability form.

At the completion of the study, the unused study drug(s) will be retained at PMRI, or returned to the Sponsor, or discarded according to Sponsor's written decision.[9]

Drug Retention

The required amount of drug units for retention will be randomly selected and documented before administering or dispensing samples. A randomizing program will be used to determine which package numbers are selected and set aside for retention purposes.[9] The process will be documented in the accountability form and then in the included in the study report.

Once the units for retention have been selected, they will be set aside in their original containers during drug receipt and maintained in closed and securely sealed bags when possible.

Further details may be provided in the Pharmacy Plan.

9.0 Study Design

9.1 Design

This is an open-label, single-dose, randomized, two-period, two-treatment, two-sequence, crossover, two-stage adaptive, BE study.

This study will employ general screening. The study will start on the day the first participant signs the study's Ethics/Institutional Review Board (ERB/IRB) approved Informed Consent Form (ICF). The end date of the clinical trial will be the last visit of the last participant. The anticipated end of study date for most participants will be their last scheduled study procedure in the Stage where they are involved. Unscheduled visits and AE follow-up may occur after the end of study procedures. The expected duration of participation in Stage 1 of this study (from voluntary signing of the ICF to the last scheduled study procedure) will be approximately 10 days.

Following completion of Stage 1, an interim analysis will be conducted to determine if Stage 2 is required. If it is required, Stage 2 screening will be completed within 30 days prior to Stage 2 first drug administration (i.e. the screening for Stage 2 can be from 30 days to 1 day before Stage 2 dosing). The duration of this study will be extended to include the total number of days required to complete Stage 1, the interim analysis, and Stage 2.

This study may be conducted in groups. The same protocol requirements and procedures will be followed for each group.

9.2 Interval Between Doses

The washout between drug administrations in Periods 1 and 2 for each participant will be at least seven (7) days (±3 hours) in Stage 1, and in Stage 2 if it is required. The washout may be extended. The duration of participation will be extended by adding on the extra washout days.

9.3 Treatments

In each period, participants will receive one (1) of the following two (2) treatments:

Table 5 Treatments

Treatment	A (Test Product)	B (Comparator Product)
Drug Product	Sapropterin dihydrochloride 100 mg/mL oral suspension	Kuvan® (sapropterin dihydrochloride) 100 mg powder for oral solution
Dose	one (1) × 10 mg/kg dose of 100 mg/mL	one (1) × 10 mg/kg dose of 100 mg
Dosing Condition	30 minutes after the start of a high-fat, high-calorie breakfast	
Dosed with		dissolved in 120±2 mL of water followed by two rinses of the dosing cup with 60±1 mL of water each time

9.4 Randomization

In Stage 1, and in Stage 2, if it is required, participants will be randomly assigned to one (1) treatment sequence according to a predetermined computer-generated randomization scheme (procedure PLAN in SAS®).

Table 6 Treatment Sequence

Sequence	Treatment	
	Period 1	**Period 2**
AB	A	B
BA	B	A

Participants will be assigned consecutive numbers in an ascending order. Each number will identify a participant and determine the sequence of drug product administration according to the randomization scheme.

9.5 Blinding

This is an open-label study. Blinding is not applicable.

Samples that are shipped to the bioanalytical laboratory will not contain treatment information on the sample label.

9.6 Sample Size

Forty-two (42) participants will be randomized in Stage 1 of this study. To ensure 42 participants participate in Stage 1, extra standby volunteers will be enrolled. These extra volunteers may not be required to participate in the study. If, however, a participant chooses to withdraw from the study for any reason prior to drug administration in Period 1, a standby participant will be used as a replacement.

If Stage 2 is required, total sample size re-estimation will be conducted after Stage 1 assuming an α level of 0.0294 and a GMR of 0.95. Based on the results of Stage 1, additional participants, including extra standby volunteers, will be enrolled.

A maximum of 100 participants will be enrolled into this study.

Volunteers who are dosed in Stage 1 cannot be dosed in Stage 2.

Volunteers who are enrolled in Stage 1, but not dosed in Stage 1, may be considered for Stage 2 (they will have to sign the consent form for Stage 2).

9.7 Study Population

The study population will consist of healthy, light-smoking (≤10 cigarettes per day) or non-smoking, male and female volunteers.

If the study is conducted in groups, participants in one (1) group will not be permitted to participate in a subsequent group.

Participants enrolled in the study will satisfy the participant selection criteria no more than 30 days prior to the first drug administration in Stage 1, or prior to the first drug administration in Stage 2 if it is required.

10.0 Participant Selection

10.1 General Screening

Recruitment into this study will initially involve PMRI recruitment procedures and general screening (Section 10.2 *Screening Procedures*), during which volunteers will voluntarily consent to and sign the ERB/IRB approved Volunteer Consent Form (VCF) and Privacy Consent Form (PCF).

An ICF specific to this study will be signed by all participants prior to any study specific procedures occurring in Stage 1 or in Stage 2, if it is required.

If a volunteer is determined to be generally healthy, additional screening procedures that are not listed in the VCF and that are specific to this study will be conducted after the participant signs the study ICF.

Volunteers who pass general screening but are not enrolled in the study will not be documented as screen failures. Participants who are enrolled in Stage 1 of the study but are not randomized are considered as withdrawn. These participants can be rescreened for Stage 2 of the study.

10.2 Screening Procedures

The following procedures will be performed at screening (within 30 days prior to the first drug administration in Stage 1, and in Stage 2 if it is required):

- Voluntary signing of the VCF and PCF;
- Inclusion/exclusion criteria and eligibility confirmation;
- Medical history;
- Demographic data collection and height and weight measurements;
- Vital signs measurements (taken after participants have been resting for at least 3 minutes in a sitting position): BP, PR, RR, and temperature;
- 12-lead ECG (recorded after participants have been resting in a semi-recumbent or supine position); and
- Blood and urine sample collection.

Table 7 Blood and Urine Tests Performed at Screening

Biochemistry	Random glucose
	Total bilirubin
	Blood urea nitrogen (BUN)
	Creatinine
	Alkaline phosphatase
	Calcium
	Lactate dehydrogenase (LD)
	Aspartate aminotransferase (AST)
	Alanine aminotransferase (ALT)
	Serum electrolytes (sodium, potassium, and chloride)

	Gamma glutamyl transpeptidase (GGT)
	Follicle-stimulating hormone (FSH) (FSH will be tested only in female participants with a menstrual history consistent with being postmenopausal)
Hematology	Complete blood count (CBC) with differential: • White blood cell (WBC) count with differential • Hemoglobin • Hematocrit • Red blood cell (RBC) count and indices • Platelet count
Serology	HIV
	Hepatitis C antibody
	Hepatitis B surface antigen
Immunohematology	Serum β-hCG (females only)
Urinalysis	Specific gravity
	pH
	Leukocytes
	Nitrites
	Protein
	Glucose
	Ketones
	Blood
	Microscopic examination ***Note:*** *microscopic examination will only be performed when any of the following urinalysis tests are reported abnormal (i.e., outside of normal ranges): leukocyte, nitrite, protein, and/or blood. The microscopic examination will be conducted on the same urine sample as the abnormal urinalysis test.*
Urine Drugs of Abuse	Amphetamines
	Barbiturates
	Benzodiazepines
	Cannabinoids
	Cocaine
	Methadone
	Opiates
	Phencyclidine
	Tricyclic antidepressants

Approximately 12 mL of blood will be collected for pre-study clinical laboratory tests.

Participants will undergo the following tests/procedures at Period 1 check-in (prior to Period 1 drug administration):

- Physical examination; and
- Remaining or follow-up screening tests.

The Investigator may require repeat clinical laboratory test(s) to determine the

participant's eligibility.

10.3 Inclusion Criteria

The following inclusion criteria will be assessed during screening (within 30 days prior to the first drug administration in Stage 1, and in Stage 2 if it is required):

1) Healthy male or female participants, light-smokers (no more than 10 cigarettes daily) or non-smokers, from 18 to 50 years of age.
2) BMI ≥18.5 and ≤30 kg/m^2 and weight ≥50.0 and ≤100.0 kg for males and ≥45.0 and ≤100.0 kg for females
3) Females may be of childbearing or non-childbearing potential:
   - Childbearing potential:
     - Physically capable of becoming pregnant
   - Non-childbearing potential:
     - Surgically sterile (i.e., both ovaries removed, uterus removed, or bilateral tubal ligation) at least three (3) months prior to first drug administration; and/or;
     - Postmenopausal (no menstrual period for at least 12 consecutive months without any other medical cause and a FSH value consistent with being postmenopausal).
4) Willing to use acceptable, effective methods of contraception.
5) Able to tolerate venipuncture.
6) Be informed of the nature of the study and give written consent prior to any study procedure.

10.4 Exclusion Criteria

The following exclusion criteria will be assessed during screening (within 30 days prior to the first drug administration) in Stage 1, and in Stage 2 if it is required:

1) History of clinically significant neurologic, hematologic, endocrine, oncologic, pulmonary, immunologic, genitourinary, psychiatric, or cardiovascular disease or any other condition which, in the opinion of the Investigator, would jeopardize the safety of the participant or impact the validity of the study results.
2) Clinically significant illness and/or surgery.
3) Known or suspected carcinoma.

4) History of hypersensitivity or idiosyncratic reaction to sapropterin dihydrochloride or any other drug substances with similar activity.

5) History of or predisposition to seizure which, in the opinion of the Investigator, would jeopardize the safety of the participant or impact the validity of the study results.

6) History of upper gastrointestinal (GI) mucosal inflammation, esophagitis, gastritis, pharyngitis, or oropharyngeal pain, which, in the opinion of the Investigator, would jeopardize the safety of the participant or impact the validity of the study results.

7) History of abuse of medicinal product or drugs within the last three (3) years.

8) History of alcohol addiction requiring treatment.

9) History or presence of alcoholism within the last three (3) years. (>40 g ethanol/day or >10 units per week [one (1) unit =150 mL of wine, or 360 mL of beer, or 45 mL of 45% alcohol])

10) History of recreational use of soft drugs (such as marijuana) within one (1) month or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within three (3) months prior to screening.

11) History of clinically significant lactose, galactose, or fructose intolerance.

12) Presence of hepatic or renal dysfunction.

13) Presence of mouth piercings (object or hole), presence of non-removable dentures and orthodontic appliances (e.g., braces, retainers), or any other alteration to the mouth that may be deemed by the Investigator to compromise drug delivery.
Note: *Dental fillings, crowns, bridges, and implants are permitted as they are not considered to compromise drug delivery.*

14) History of malabsorption within the last year or presence of clinically significant gastrointestinal (GI) disease.

15) Presence of a medical condition requiring regular medication (prescription and/or over-the-counter) with systemic absorption.

16) Positive serology test results for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, or human immunodeficiency virus (HIV)-1 and HIV-2 antibodies at screening.

17) Positive test result for urine drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine, and tricyclic antidepressants).

18) Difficulty fasting or consuming high-fat, high-calorie or standard meals.

19) Regularly smokes more than 10 cigarettes per day within 6 months prior to the first drug administration.

20) Participants who received an implanted or injected (depot injection) medication, including hormonal contraceptives, within three (3) months prior to the first drug administration until the study is complete.

21) Females who:
- Have discontinued or changed the use of implanted, intrauterine, intravaginal, or injected hormonal contraceptives within six (6) months prior to the first drug administration until the study is complete;
- Have discontinued or changed the use of oral or patch hormonal contraceptives within one (1) month prior to the first drug administration until the study is complete;
- Are pregnant (serum β-hCG consistent with pregnancy), planning on becoming pregnant during the study or within four (4) weeks after the end of the study; or
- Are breast-feeding.

22) Donation or loss of whole blood (including clinical trials):
- ≥50 mL and <500 mL within 30 days prior to the first drug administration; or
- ≥500 mL within 56 days prior to the first drug administration.

23) Donation of plasma within seven (7) days prior to dosing.

24) Participation in a clinical trial that involved administration of a biological product within 90 days prior to the first drug administration, or recent participation in a clinical investigation that, in the opinion of the Investigator, would jeopardize participant safety or the integrity of the study results.

25) Participation in a clinical trial that involved administration of an investigational medicinal product, marketed drug or device within 30 days prior to the first drug administration, or recent participation in a clinical investigation that, in the opinion of the Investigator, would jeopardize participant safety or the integrity of the study results.

26) On a special diet within 30 days prior to the first drug administration (e.g., liquid, protein, raw food diet).

27) Have had a tattoo or body piercing within 30 days prior to the first drug administration.

28) Have clinically significant findings in vital signs measurements and systolic blood pressure (SBP) <90 mmHg or >150 mmHg and diastolic blood pressure (DBP) <50 or >90 mmHg, or PR <50 or >100 bpm). Vitals signs may be repeated up to two (2) times, to determine if the values are significantly abnormal.

29) Have clinically significant findings in a 12-lead ECG. ECG readings may be repeated up to two times, to determine if the values are significantly abnormal.

30) Have clinically significant abnormal laboratory values. Laboratory tests may be repeated up to two (2) times, to determine if the values are significantly abnormal.

31) Have significant diseases.

32) Use of any of the following drugs within 30 days prior to drug administration:
- Breast cancer resistance protein (BCRP) substrates (e.g., rosuvastatin);
- Drugs affecting nitric oxide-mediated vasorelaxation (e.g., PDE-5 inhibitors, sildenafil, vardenafil, or tadalafil);
- Drugs which alter GI pH/movement (e.g., omeprazole, ranitidine)
- Folate synthesis inhibitors (e.g. methotrexate, valproic acid, phenobarbital, or trimethoprim);
- Levodopa; or
- St. John's wort.

33) Use of vaccine including COVID-19 vaccine within 14 days prior to the first drug administration until the study is complete.

34) Vulnerable participants.

35) If Stage 2 is required, participants dosed in Stage 1.

The following exclusion criteria will be assessed at Period 1 check-in or prior to Period 1 drug administration:

36) Have clinically significant findings from a physical examination.

10.5 Effective Methods of Contraception

Participants in this study must use an acceptable single- or double-method of contraception as defined below:

- Females: from 21 days prior to the first drug administration in Stage 1, and in Stage 2 if it is required, until at least 28 days after the last drug administration in each stage;
- Males: from the first drug administration in Stage 1, and in Stage 2 if it is required, until at least 28 days after the last drug administration in each stage.

Table 8 Acceptable Effective Methods of Contraception

Single-Method	Double-Method (must use at least two)	
Surgically sterile	Diaphragm with Spermicide	Non-hormonal IUD
Postmenopausal	Cervical cap with Spermicide	Vasectomy
Remain abstinent	Condom with Spermicide	Hormonal contraception
Hormonal contraception includes: Oral contraceptives, intra-vaginal devices, hormonal IUD, injections, transdermals, and implants		

Male participants will be instructed not to donate sperm from the first drug administration in Stage 1, and in Stage 2 if it is required until at least 28 days after the last drug administration in each stage.

11.0 Study Procedures

11.1 Order of Priority

Top priority is given to drug administration at 0-hour – it will be completed at the time scheduled for each participant.

Priority for completion of study procedures that are scheduled to occur at the same time is given to:

1. PK blood collection
2. Vital signs measurements.
3. 12-lead ECG recording.
4. Meal administration.

These procedures are not required to be conducted in priority order when they can be completed within their scheduled window allowance (±X min).

Table 9 Scheduled Windows of Allowance for Study Procedures

Procedure	Timepoint	Acceptable Window from Scheduled time
Vital signs measurements	Pre-dose (0-hour)	Within 180 minutes prior to dosing
	>0-hour	±20 minutes
12-lead ECG recording	Check-in	From check-in to dosing
	>0-hour	±30 minutes

Procedure	Timepoint	Acceptable Window from Scheduled time
PK blood collection	-1 hour and -0.5 hour	±5 minutes
	Pre-dose (0-hour)	Within 10 minutes prior to dosing
	>0-hour to 10 hours	±1 minute
	>10 hours	±3 minutes

11.2 Restrictions

The following restrictions apply to this study. The participants will be provided medication guidelines at screening that informs about general restrictions that would apply to this study. Compliance with restrictions will be documented at each clinic visit (screening check-in returns).

Table 10 Study Restrictions

Restriction	Prior to Drug Administration	Until
Medication (prescription or over-the-counter) *Exceptions will be made for:* • *Hormonal contraceptives;* • *Non-systemic and/or topically applied products (prescription or otherwise); and* • *The occasional use common analgesics*	14 days	Last blood draw of last period
Herbal/natural products	14 days	Last blood draw of last period
Oral and injectable nutritional supplements and vitamins	14 days	Last blood draw of last period
Food or beverage containing grapefruit, Seville orange, starfruit, pomegranate, pineapple, or pomelo	7 days	Last blood draw of last period
Alcohol and alcohol-containing products	48 hours	Last blood draw of each period
Caffeine- and xanthine-containing products	48 hours	Last blood draw of each period
Food containing poppy seeds	24 hours	Last blood draw of each period
Nicotine Products	Throughout confinement. During the washout period, no more than 10 cigarettes are permitted per day and no other nicotine products will be allowed.	
Chewing Gum	Throughout Confinement	
Strenuous Activity	Throughout Confinement	

Adherence to the restrictions will be confirmed and recorded at each check-in. Participants who do not comply with these restrictions will be assessed by the Investigator/PK Scientists regarding their continued participation in the study.

11.3 Housing

Participants will be confined in-house from at least 10.5 hours prior to drug administration until at least 24 hours post-dose in each period. Smoking breaks will not be allowed during confinement.

11.4 Tests/Procedures at Check-in

At Period 1 check-in, participants will voluntarily sign the ICF.

Participants will have their weight measured at Period 1 check-in for dose calculation.

Participants will undergo the following tests/procedures at every period check-in:

- Confirmation of ongoing eligibility based on procedures completed at each period check-in;
- Temperature measurement;
- 12-lead ECG recording (from check-in to dosing);
- Urine drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine, and tricyclic antidepressants);
- Urine and serum β-hCG (females only);
- Breath alcohol; and
- AE and concomitant treatment collection.

At check-in, the delegated staff will document in the source records that all eligibility criteria have been met by the participants (exclusion/inclusion criteria and restrictions reported in ICF).

Approximately five (5) mL of blood will be collected for serum β-hCG tests at each check-in (five [5] mL at the check in of Period 1 and 5 mL for Period 2 for a total of 10 mL for each participant).

This is valid both for Stage 1 and Stage 2.

Clinical study personnel reserve the right to conduct additional testing (e.g.,breath alcohol test, diagnostic imaging) at any time throughout the study.

11.5 Administration of Food and Fluid

After an overnight fast of at least 10 hours, participants will consume a high-fat, high-calorie breakfast starting 30 minutes prior to drug administration.

Table 11 High-Fat, High-Calorie Breakfast

	Calories (kcal)	Total Fat (g)	Carbohydrates (g)	Protein (g)
2 eggs	140	10	0	12
10 g of butter for cooking eggs	70	8	0	0
2 slices of whole wheat toast (approximately 64 g)	160	2	31	7
10 g of butter for toast	70	8	0	0
3 triangle pieces of hash brown potatoes (approximately 130 g)	218	14	23	2
250 mL of whole milk	160	8	12	8
3 strips of chicken bacon (approximately 63 g)	105	7	0	9
Total weight (g)		57	66	38
Total calories (kcal)	923	514	262	152
Relative caloric content		56%	28%	16%

Note: *Due to supply chain issues, some ingredients may not be available therefore, the content of the high-fat, high-calorie breakfast is subject to change. Any change will be notified to the Sponsor and ensured that the total caloric content and breakdown between fat, carbohydrate, and protein is maintained according to FDA requirements: high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories). The meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.*

Participants who do not consume the entire meal in 30 minutes or less will be dismissed from the study prior to drug administration, unless otherwise specified by the Investigator or Sponsor.

Participants will fast for at least four (4) hours post-dose.

Standardized meals will be provided throughout confinement. The meals will be identical for both periods.

Water will be allowed as desired except for one (1) hour before and one (1) hour after drug administration (except for the water administered with Treatment B).

11.6 Study Drug Administration

Treatment A (Test Product)

Prior to drug administration, participants will be instructed not to spit out the study drug. Participants who are non-compliant will be removed from the study.

1) Participant ID will be verified by checking the participant's armband against the label on the syringe(s) of the study drug, the drug administration dosing log, and the master list.
2) Study treatment and volume will be verified and documented on the drug administration dosing log.
3) The participant's mouth will be checked for the presence of mouth piercings (i.e., object or hole), non-removable dentures, orthodontic appliances (e.g., braces, retainers), or any other alteration to the mouth that may compromise drug delivery.
4) The participant will be instructed to sit with their head tilted slightly upwards. Delegated staff will double check the syringe label(s) with each participant's ID prior to the scheduled drug administration time.
5) At the scheduled time, the delegated staff will place the dosing syringe into the participant's mouth at an angle and the upper portion of the dosing syringe (not just the tip) will be inserted into the participant's mouth. The participant will be asked to close their mouth tightly around the dosing syringe.
6) At the time of drug administration, the drug will be administered with the syringe by the delegated staff directly into the participant's mouth.
7) The participant will be asked to immediately swallow the suspension in their mouth.
8) Procedures five (5) to seven (7) will be followed for dosing with additional syringes.
9) The time of dosing will be considered as the time the first syringe of drug is injected into the participant's mouth. The actual time of administration will be reported in the drug administration form. Drug administration staff will confirm that the dosing is performed within 15 minutes from the drug preparation time.
10) Administration of the study drug to each participant will be properly documented on the eCRF by the delegated staff and will be within 15 minutes of drug preparation.
11) After the participant swallows all of the drug, a hand and mouth check will be immediately performed by the dosing assistant to ensure that the study drug has been completely swallowed.
12) The used syringe(s) will be returned by the delegated staff to the pharmacy before being inspected and discarded.

If any deviations occur during drug administration, the Sponsor will be notified by PMRI.

Treatment B (Comparator Product)

Prior to drug administration, participants will be instructed not to touch, swirl, or spit out the study drug. Participants who are non-compliant will be removed from the study.

1) Participant ID will be verified by checking the participant's armband against the label on the study drug container, the dosing log, and the master list.
2) Study treatment and volume will be verified and documented on the dosing log.
3) The participant's mouth will be checked for the presence of mouth piercings (i.e., object or hole), non-removable dentures, orthodontic appliances (e.g., braces, retainers), or any other alteration to the mouth that may compromise drug delivery.
4) The participant will be instructed to sit with their head tilted slightly upwards.
5) At the designated dosing time the participant will be given the solution (drug powder mixed in 120± 2 mL of water) to drink.
6) To ensure that the entire dose is consumed, the dosing cup will be rinsed twice with an additional 60± 1 mL of room-temperature water each time (total volume of 120± 2 mL) and the contents will be swallowed.
7) The dosing cup will be discarded after inspection by the delegated staff.
8) A hand and mouth check will be immediately performed by the dosing assistant to ensure that the drug and water have been completely swallowed.
9) The actual time of administration will be reported in the drug administration form, with a comment confirming that the dosing is performed 15 minutes from the drug preparation time.

If any deviations occur during drug administration, the sponsor will be notified by PMRI.

11.7 Concomitant Treatment

The Investigator may request the administration of concomitant drug or non-drug treatment for participants.

If drug therapy other than the permitted medication (see Table 10) is required during the study, the decision whether to continue or discontinue participation in the study will be made by the Investigator/PK Scientists.

Clinic staff may also administer non-drug treatment(s) that do not deviate from the procedures outlined in the protocol (e.g., ice pack, allowing the participant to lie down). All treatment(s) provided will be documented.

11.8 Posture and Physical Activity

Participants will:

- Remain seated for six (6) hours following drug administration (unless required to ambulate for study specific procedures or use the restroom) and may resume normal activity thereafter;
- Not engage in any strenuous activity throughout confinement; and
- Be permitted to lie down if they experience drowsiness, dizziness, or other AE requiring such a position.

11.9 Health Status Monitoring

The Investigator will be on-site prior to drug administration and until four (4) hours after the last participant is dosed. The Investigator will remain on-call until the end of the study.

Participants will be questioned regarding their health status throughout the study.

Additional health monitoring procedures may be conducted at any time if deemed necessary by the Investigator.

11.10 Vital Signs Measurements

Temperature will be measured daily during confinement, regardless of participation status while in the clinic.

Vital signs (BP and PR) will be measured after the participants have been resting for at least three (3) minutes in a sitting position at pre-dose (0-hour) and at three (3) and five (5) hours post-dose.

Additional measurements will be taken if requested by the Investigator.

11.11 Electrocardiogram Monitoring

Electrocardiograms will be recorded after the participants have been resting in a semi-recumbent or supine position pre-dose (0-hour) and at four (4) and six (6) hours post-dose.

Additional recordings will be taken if requested by the Investigator.

11.12 Pharmacokinetic Sample Collection

In each period, 22 samples are scheduled to be collected:

Pre-dose at (-1, -0.5, and 0-hour) and at 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 12, 16, 20, and 24 hours after drug administration

Samples collected outside the acceptable window indicated in Table 9 will not be considered as protocol deviations; the actual clock time of each sample collection will be recorded.

The PMRI PK Scientists will be contacted to evaluate continued participation in the study if any of the following incidents occur in any period:

- The 24-hour scheduled blood draw is missed; or
- At least two (2) consecutively scheduled blood draws are missed.

The impact of these incidents on the estimation of PK parameters will be made as close to the time of occurrence as possible.

Samples will be collected in labeled four (4) mL blood collection tubes and processed as per the lab manual.

Blood will be collected by direct venipuncture or from an indwelling cannula, which will be placed in an arm vein of the participant.

Approximately 175 mL of blood will be collected from each participant over the entire study for PK analysis.

11.13 Sample Processing, Storage and Shipment

Samples will be protected from UV light at all times during collection and processing.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in the laboratory manual.

11.14 End-of-Study Procedures

End-of-study for each participant is defined as the last study specific procedure in the last period of the study or the last study specific procedure prior to the discontinuation of a participant from the study.

End-of-study will include the following procedures:

- A physical examination;
- Vital signs (taken after participants have been resting for at least three (3) minutes in a sitting position): BP, PR, RR, and temperature;
- 12-lead ECG (recorded after the participants have been resting in a semi-recumbent or supine position); and
- Blood and urine sample collection.

Table 12 Blood and Urine Tests Performed at End-of Study

Biochemistry	Random glucose
	Total bilirubin
	BUN
	Creatinine
	Alkaline phosphatase
	Calcium
	LD
	AST
	ALT
	GGT
	Serum electrolytes (sodium, potassium, and chloride)
Hematology	CBC with differential: • WBC count with differential • Hemoglobin • Hematocrit • RBC count and indices • Platelet count
Immunohematology	Serum β-hCG (females only)
Urinalysis	Specific gravity
	pH
	Leukocytes
	Nitrites
	Protein
	Glucose
	Ketones
	Blood
	Microscopic examination ***Note:*** *microscopic examination will only be performed when any of the following urinalysis tests are reported abnormal (i.e., outside of normal ranges): leukocyte, nitrite, protein, and/or blood. The microscopic examination will be conducted on the same urine sample as the abnormal urinalysis test.*

Approximately eight (8) mL of blood will be collected for post-study clinical laboratory tests. The Investigator may require repeat clinical laboratory test(s).

For participants who are removed or withdrawn from the study, end-of-study procedures will still be conducted. Participants who do not return for end-of-study procedures will be considered lost-to-follow-up.

If a participant is not able to repeat any end-of-study tests at the clinic, they may repeat the test with their local healthcare provider/laboratory. All external results will be reviewed by the Investigator.

Participants will not be allowed to participate in another clinical research study until 30 days after their last drug administration or 30 days after their last PK blood collection, whichever is longer.

12.0 Criteria for Removal from the Study

Participants will be free to voluntarily withdraw at any time, for any reason, or they may be dismissed, if necessary, to protect their health or the integrity of the study.

Participants who experience vomiting within 10 hours after drug administration will be removed from the study.

Participants who experience vomiting after this time will be assessed for their continued participation in the study by the Investigator.

Participant(s) may be removed from the study for any of the following reasons:

- Positive drug screening, or alcohol test;
- Positive pregnancy test;
- AE(s), SAE(s), or other health issue assessed by the Investigator, including an undisclosed pre-existing condition;
- If the Investigator judges it is in the participant's best interest;
- Administrative reasons (e.g., uncooperative, non-compliant); or
- Missing sample(s) or incidents of AEs that affect the PK of the drug/analyte.

The reason for removal of participants will be recorded and reported. Any participant who discontinues for the above reasons if not already dosed for Period 1 will not be replaced.

Participants who withdraw or are dismissed from the study during confinement will be asked to remain in the clinic for safety monitoring until released by the clinic staff. The health status of these participants will continue to be documented.

12.1 Use of Data for Discontinued Participants/Missing Samples

Data that was obtained prior to a participant's discontinuation in this study will be maintained, used, and included in subsequent analyses, as applicable.

Missed blood samples will be documented as deviations when a participant has one or more missing samples (e.g., due to incorrect processing or delayed sampling).

13.0 Adverse Events

Pharma Medica Research Inc. has established SOPs in conformity with regulatory requirements to ensure the timely, accurate, and complete reporting of safety information. These SOPs will be followed during the conduct of this study.

13.1 Adverse Event Definition and Classification

13.1.1 Adverse Event

An AE is defined as any untoward medical occurrence in a participant administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. A treatment emergent AE (TEAE) can, therefore, be any unfavorable and unintended sign (including abnormal laboratory finding), symptom or disease temporarily associated with the use of an investigational medicinal product (IMP), whether or not related to the IMP.

A non-treatment emergent adverse event (NTEAE) is defined as any acute existing disease or symptom, which occurs during and after participants sign the ICF until first administration of the investigational medicinal product (IMP). Non-treatment emergent AEs will be reported separately from TEAEs in the study report. If, after administration of the IMP, a deterioration of the severity of the existing NTEAE is observed, this sign or symptom will be documented as a TEAE.

13.1.2 Serious Adverse Event

A serious adverse event (SAE) is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening (i.e., its occurrence places the participant at immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Is otherwise considered to be an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Medical and scientific judgement should be exercised in deciding whether an event

should be considered as an Important Medical Event. Examples of such medical events include:

- Allergic bronchospasm requiring intensive treatment in an emergency room or at home;
- Blood dyscrasias or convulsions that do not result in hospitalization;
- Development of drug dependency or drug abuse.

13.1.3 Severity

Adverse events will be classified according to the following severity scale:

Table 13 Severity Scale for Adverse Events

Severity	Description
Mild	Awareness of signs and symptoms, but are easily tolerated; are of minor irritant type; causing no limitations of usual activities. Signs or symptoms may require minor action.
Moderate	Discomfort severe enough to cause some limitations of usual activities and may require action.
Severe	Incapacitating with inability to carry out usual activities or significantly affects clinical status, and requires specific action and/or medical attention.

13.1.4 Relationship to Study Drug

Adverse events will be assessed for the relationship to the IMP (causality) according to the following scale:

Table 14 Scale to Determine Adverse Event Relationship to Study Drug

Category	Description
[Related] Probable (must have first three points)	This category applies to AEs that are considered, with a high degree of certainty, to be related to the investigational product. An AE may be considered probable, if: 1. It follows a reasonable temporal sequence from the administration of the study treatment. 2. It cannot be reasonably explained by the known characteristics of the participant's clinical state, environmental or toxic factors or other modes of therapy administered to the participant. 3. It disappears or decreases on cessation or reduction in dose (there are important exceptions when an AE does not disappear upon discontinuation of the study treatment, yet relatedness clearly exists; e.g. (1) bone marrow depression, (2) tardive dyskinesias).

	4. It follows a known pattern of response to the suspected study treatment. 5. It reappears upon re-challenge.
[Related] Possible (must have first two points)	This category applies to AEs in which the connection with the investigational product administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when: 1. It follows a reasonable temporal sequence from the administration of the study treatment. 2. It may have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It follows a known pattern of response to the suspected study treatment.
Remote/Unlikely (must have first two points)	In general, this category is applicable to an AE that meets the following criteria: 1. It does not follow a reasonable temporal sequence from the administration of the study treatment. 2. It may readily have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It does not follow a known pattern of response to the suspected study treatment. 4. It does not reappear or worsen when the study treatment is re-administered.
Unrelated	This category is applicable to AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.), and do not meet the criteria for medication relationship listed under remote/unlikely, possible, or probable.

Drug-related AEs will be defined as AEs with (related) possible or probable relationship to the study drug in the study report.

13.2 Procedures for Collecting Adverse Event Information

Prior to subsequent drug administration(s), participants will be questioned concerning unusual symptoms that may have occurred since the previous administration of the study drug(s).

Any AEs, whether serious or non-serious, will be monitored throughout the study and followed to resolution, when possible, regardless of whether the participant is still participating in the study.

Participants will be instructed to inform clinic personnel of AEs that may arise during the course of the study.

Symptoms will be recorded and will be reviewed by the Investigator prior to any subsequent drug administration.

When appropriate, medical tests and examinations will be performed to document resolution of the event(s).

All AEs should be given appropriate medical care. Treatment may include one of the following:

- None;
- Non-drug therapy;
- Drug therapy;
- Drug therapy and non-drug therapy; or
- Not available.

The action taken toward the IMP will be documented, and will be assigned to one of the following categories, in accordance with Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) standards:

- Dose not changed;
- Dose increased;
- Dose rate reduced;
- Dose reduced;
- Drug interrupted;
- Drug withdrawn;
- Not applicable; or
- Unknown.

In addition, the outcome of the AE will also be documented, and assigned to one of the following categories, per CDISC SDTM standards:

- Not recovered/not resolved;
- Recovered/resolved;
- Recovered/resolved with sequelae;
- Recovering/resolving;
- Fatal; or
- Unknown.

13.3 Procedures for Reporting Adverse Events

Adverse events will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA) and reported with respect to severity, duration, relationship to study drug(s) and action taken.

All SAEs, whether deemed drug-related or not, will be reported to the Sponsor immediately (within 24 hours) after the occurrence, followed by a written report

within five (5) working days. PMRI will be responsible for notifying Health Canada.

For reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs) by the PMRI to Health Canada, the following timelines apply:

Table 15 Timelines for Reporting SUSARs

SUSAR	Expedited Reporting Timelines
Neither fatal nor life-threatening	Within 15 calendar days after becoming aware of the information
Fatal or life-threatening	Within seven (7) calendar days after becoming aware of the information, followed by a comprehensive report within eight (8) calendar days after informing Health Canada

Adverse event reports that are expected or unexpected, but not serious, should not be reported to Health Canada, but rather monitored and tracked by the Sponsor. PMRI will report to Health Canada "expected, serious" AEs, where an increase in the rate of occurrence or severity, is judged to be clinically important.

The following Sponsor representative is to be contacted immediately following the occurrence of an SAE:

Patrizia Mesiti
Regulatory Affairs Pharma Manager
and Head of Pharmacovigilance
APR Applied Pharma Research s.a.
Via Corti 5
CH - 6828 Balerna
Switzerland
Email: apr.vigilance@apr.ch

13.4 Pregnancy

In the event a dosed female participant or the female partner of a dosed male participant (i.e., has received at least one dose of the investigational product) becomes pregnant during or shortly after participation in the study, this pregnancy will be reported to the Sponsor (or representative) within 24 hours of first knowledge of the event. Any participant who becomes pregnant during the study will be immediately withdrawn.

Follow-up information regarding the course and outcome of the pregnancy will be documented (after obtaining the consent of the female partner) as per site's SOP. If the outcome of the pregnancy meets the criteria of reportable event, reporting of

the event to the Institutional Ethics Committee responsible for the study and/or to applicable regulatory agency(ies) will be performed as per site's SOP.

14.0 Data Sources, Electronic Case Report Forms, and Data Management

14.1 Data Sources

Data sources will include clinical site record documents (study forms), laboratory reports, electronic source data capture applications, and ECG strips.

As part of the responsibilities assumed by conducting the study, the Investigator agrees to maintain adequate documentation for participants enrolled as part of the clinical investigation of this protocol. The Investigator agrees to maintain accurate and complete source documentation for each participant.

Pharma Medica Research Inc. ensures that direct access to all source data/documents and eCRF/CRF regarding the study will be granted upon request from Sponsor, Sponsor representatives, monitor, auditor, and applicable authorities.

14.2 Electronic Case Report Forms and Data Management

TrialMaster®, a validated US FDA 21 CFR Part 11 compliant eCRF system from vendor Anju Software, Inc., will be used to capture data for the study. The eCRF will be designed to capture the data according to the protocol, Sponsor requirements, and CDISC CDASH guidelines.

Data will be transcribed directly from the clinic source records into the eCRF system. External data (e.g., clinical laboratory data) and electronic source data will be imported into the eCRF system from electronic transfer files.

Medical coding will be done within the eCRF system. Adverse events and medical history will be coded using the MedDRA. Concomitant medications will be coded using the WHODrug dictionary.

Serious AE reconciliation, as applicable, will be performed as described in the SAE Reconciliation Plan.

Quality control and validation processes will be implemented to ensure completeness, accuracy, consistency, and integrity of the data from database development through to database lock. If any changes are made within the eCRF, a full audit trail will be completed.

The Investigator is responsible for the final review, approval, and sign-off of all the eCRFs for the study.

Database lock will be conducted after all data reviews have been completed, all queries have been resolved and appropriate approvals have been obtained.

Data management processes will be described in detail in the Data Management Plan).

15.0 Dataset Definitions

15.1 Safety Dataset

The safety dataset will include participants who receive at least one (1) administration of any study treatment. Data from participants in this dataset will be used for the assessment of safety.

15.2 Pharmacokinetic Dataset

Data from the following participants will be included in the PK dataset:

- Participants from whom the estimation of C_{max} and/or AUC parameters will be possible for both periods; and
- Participants who complied with all protocol requirements or encountered protocol deviations that do not impact the estimation of the PK parameters.

The PK dataset will be defined prior to the assay of samples. However, if a sufficient reason to exclude a participant's data is identified during the analysis (i.e. suspected non-compliance, insufficient quantifiable data, etc.) the participant's data may be excluded from the PK dataset at the discretion of the PK Scientist. Data from participants in this dataset will be used for the PK analysis. If deviations occur, they will be assessed for their impact on the PK dataset and will be included in the study report.

15.3 Statistical Dataset

The statistical dataset will include all data in the PK dataset modified by the following occurrences:

- Individual period data for the affected analyte will be excluded for a participant who exhibits lack of any measurable concentrations or only very low plasma concentrations of the analyte for the Test or Comparator Product in a given period. A participant is considered to have very low plasma concentrations if the AUC is less than 5% of the Test or Comparator Product geometric mean AUC (which should be calculated without inclusion of data from the outlying participant); and
- Individual period data will be excluded if a pre-dose concentration greater than 5% of the corresponding C_{max} is observed for a participant in a given period.

If none of these exclusions are met, the statistical dataset will be identical to the PK dataset. Data from participants in this dataset will be used for the statistical analysis.

16.0 Analytical Procedures

16.1 Samples to be Assayed

All samples obtained from all participants will be sent to Syneos according to the instructions detailed in the lab manual and assayed.

The data will not be released for PK and statistical analyses until after all samples of each stage have been analyzed by the bioanalytical laboratory.

16.2 Analyte(s) in Biological Matrix

Plasma samples will be assayed for sapropterin using a validated analytical method according to the principles of Good Laboratory Practice (GLP) (2013).[9,10]

16.3 Analytical Retesting and Aberrant Values

The validity of all assayed values will be based on the rules detailed in the bioanalytical laboratory SOPs. If unacceptable values are determined to be attributable to analytical reasons, the samples will be re-assayed according to the bioanalytical laboratory SOPs and the guidelines set by regulatory authorities. All cases of re-assay will be reported in the analytical report.

16.4 Incurred Sample Reanalysis

Incurred sample reanalysis will be conducted on 10% of 1000 assayed samples and at least 5% of the remaining assayed samples to demonstrate reproducibility of the analytical method. The concentration levels from this reanalysis will not be used for the PK and statistical evaluations.

17.0 Data Evaluation

17.1 Pharmacokinetic Analysis

Pharmacokinetic analysis will be performed on available data from participants in the PK dataset.

Sapropterin is an endogenous compound and therefore it is to be analyzed both with and without baseline correction. Pharmacokinetic parameters will be calculated based on uncorrected plasma concentrations and baseline-corrected plasma concentrations, as appropriate. Baseline corrected for sapropterin will be performed by calculating the mean plasma pre-dose levels for each participant within each period of the study using the measured concentrations at the -1, -0.5, and the 0-hour time points. The mean of these pre-dose values will be the *baseline* for that period for that participant. The baseline value will be subtracted from all subsequent measured concentrations (including 0-hour) for the same participant within the same period. Negative baseline adjusted levels will be set to zero before the PK analysis. No adjustment of the pre-dose or post-dose concentrations will be performed for the baseline uncorrected data.

The actual post-dose sample collection times will be used in the PK analysis.

The following PK parameters/observations will be estimated for uncorrected and baseline-corrected sapropterin using a non-compartmental approach in Phoenix™ WinNonlin®:

AUC_t:	The area under the analyte concentration versus time curve, from time zero (0) to the time of the last measurable analyte concentration (t), as calculated by the linear trapezoidal method.
AUC_{inf}:	The area under the analyte concentration versus time curve from time zero to infinity. $AUC_{inf} = AUC_t + C_t/K_{el}$, where C_t is the last measurable analyte concentration.
C_{max}:	Maximum measured analyte concentration over the sampling period.
T_{max}:	Time of the maximum measured analyte concentration over the sampling period.
T_{lag}:	Time of observation prior to the first observation with a measurable (non-zero) concentration
K_{el}:	The apparent first-order elimination rate constant.
T_{half}:	The apparent elimination half-life.

Cl/F:	Apparent clearance
V_z/F:	Apparent volume of distribution
AUC_t/AUC_{inf}:	The ratio of AUC_t to AUC_{inf}.
Residual Area:	$(AUC_{inf} - AUC_t)/AUC_{inf}$.
TLIN:	Start time for linear regression.
LQCT:	Last quantifiable concentration time.
R^2:	Coefficient of determination obtained from regression analysis.

K_{el}, T_{half}, and AUC_{inf} parameters will not be estimated for concentration-time profiles where the terminal linear phase is not clearly defined.

Individual and mean plasma concentration versus time curves will be plotted.

Additional PK analyses may be conducted if deemed appropriate. A complete description of the PK analysis to be performed will be provided in the Statistical Analysis Plan (SAP).

17.2 Interim Analysis, Criteria for Initiation of Stage 2 and Criteria for Evaluation:

An analysis on AUC_t, AUC_{inf}, and C_{max}[1] will be performed after the 42 participants have completed Stage 1. Estimates of the within-subject variance for these parameters will be obtained. The power will be estimated using this variance estimate from Stage 1, assuming an α level of 0.05 and a geometric mean ratio (GMR) of 0.95.

- If all power estimated from AUC_t, AUC_{inf}, and C_{max} are greater than or equal to 80%, a 90% (α=0.05) CI for the AUC_t, AUC_{inf}, and C_{max} true GMR and a point estimate will be calculated. The bioequivalence criteria will be evaluated based on the results of analysis from Stage 1 where the 90% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR has to fall completely between 80.00 and 125.00%. Regardless of the outcome of the BE evaluation, Stage 2 will not be required, and the study will be considered complete with the participants from Stage 1 only and a conclusion on bioequivalence will be made.
- If at least one power estimated from AUC_t, AUC_{inf}, and C_{max} is less than 80%, a 94.12% (α=0.0294) CI for the AUC_t, AUC_{inf}, and C_{max} true GMR will be calculated. If the results of the analysis from Stage 1 indicate that the 94.12% CI for the AUC_t, AUC_{inf}, and C_{max} true GMR falls completely between 80.00 and 125.00%, Stage 2 will not be required and the study will be considered complete with the participants from Stage 1 only. If the results from Stage 1

indicate that the 94.12% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR does not fall completely between 80.00 and 125.00%, Stage 2 will be initiated.

- If BE bioequivalence is not demonstrated, the study can continue with Stage 2. The sample size for Stage 2 is to be estimated using the variance at Stage 1 and an α level of 0.0294. The BE is evaluated at Stage 2 using data from both stages (α=0.0294) where the 94.12% CI for the C_{max}, AUC_t, and AUC_{inf} true GMR has to fall completely between 80.00 and 125.00%.

17.3 Statistical Analysis

Statistical analysis will be performed on quality assured data, with unbalanced sequences if necessary, from participants in the statistical dataset. Different statistical models may be applied for 2 stages, e.g., stage effect to be considered in Stage 2.

Analysis of Co-Variance (ANCOVA) will be applied for baseline-corrected analysis on log-transformed AUC_t, AUC_{inf}, and C_{max} parameters, using the actual total dose included in the ANCOVA statistical model as a covariate. The ratio of geometric least square means for treatments and the corresponding confidence interval (CI) for the ratio of geometric least square means, based on least-squares means from the ANCOVA of the log-transformed data, will be calculated for AUC_t, AUC_{inf}, and C_{max}.

Uncorrected and baseline-corrected data will be presented.

Details of statistical analysis will be included in the SAP.

Additional statistical analyses may be conducted if deemed appropriate. For example, an analysis with potency corrected data will be completed (to adjust the ratio and CI for the comparison between Test and Comparator Products). Potency correction will be done if the difference in assay is >5%. Potency correction will be accomplished by dividing the relevant PK parameters by the reported potency value for that specific treatment.[11]

18.0 Safety Evaluation

There will be no formal statistical evaluation of safety or tolerability. An assessment of safety will be based primarily on the incidence, frequency, and severity of AEs. Adverse events will be tabulated by treatment and participant number for all participants in the safety dataset.

19.0 Investigator and Ethical Considerations

19.1 Basic Principles

This research will be carried out in accordance with GCP as set out by the International Council for Harmonisation (ICH), the basic principles defined in the U.S. Code of Federal Regulations (21 CFR Part 312), including 21 CFR 312.120, and the World Medical Association Declaration of Helsinki (Helsinki, Finland, October 2024) and in accordance with all national, state/provincial, and local laws and regulations.

The Clinical Trial Application (CTA) for the study will be reviewed by Health Canada and the study drug will not be administered unless an appropriate 'No Objection Letter' has been received.

A CTA notification will be provided to Health Canada upon completion of the study or at study termination.

19.2 Investigator Responsibilities

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and applicable regulatory requirements.

The Investigator can delegate tasks to qualified designates. All personnel involved in the conduct of this study must be qualified by education, training, and experience to perform their assigned responsibilities.

Administrative items/documents are meant to guide the Investigator in the conduct of the study but may be subject to change based on industry and government regulations, SOPs, working practice documents, or guidelines.

19.3 Ethical Review

A study will not be initiated by PMRI without written approval from an ERB/IRB (https://optimumerb.com).

This protocol, the ICF, advertisements to be used for the recruitment of study participants (if applicable), and any other written information regarding this study to be provided to the participants, already agreed with the Sponsor, will be reviewed and approved by the ERB/IRB prior to the initiation of the study or use of the material.

The general PCF and VCF are not specific for the study. They are prepared by PMRI and Sponsor approval is not requested. They are reviewed and approved

by the ERB/IRB prior to participant signing, along with generic advertisements that will be used for the recruitment of participants (if applicable).

Any changes to the ICF, or use of supplemental ICFs in this study, will be reviewed and approved by the ERB/IRB prior to their implementation.

Documentation of all ERB/IRB approvals and copies of all correspondence with the Competent Authority/EC/IRB regarding this study will be maintained by PMRI and provided to the Sponsor promptly after obtaining them. They will be included in the Investigator Trial Master File by PMRI.

A copy of the ERB/IRB's approval documentation will be included in the final report.

A summary of the study will be provided to the ERB/IRB by PMRI regulatory staff upon completion of the study or at least on a yearly basis.

The ERB/IRB is constituted and operates in accordance with Division 5 of the Canadian Food and Drug Regulations, ICH/GCP Guidelines, FDA 21 CFR Parts 50 and 56, Department of Health and Human Services (DHHS) Section 45 CFR 46, Declaration of Helsinki, FDA Information Sheets: Guidance for IRBs and Clinical Investigators, and the Tri-Council Policy for Ethical Conduct of Research Involving Humans.

19.4 Participant Consent (ICF)

ICF written consent in compliance with local legislation and regulations shall be obtained from each volunteer before any study-specific procedures are started. Additionally, each volunteer will be provided with verbal and written information, in non-technical terms, which describes the nature and duration of the study. Adequate information will be provided to volunteers in order for them to make an informed decision regarding his/her participation.

If the ICF is revised during the course of the study, all active participants must voluntarily consent to and sign and date the revised form. A copy of any revised consent form approved by the ERB/IRB will be provided with the final report.

Prior to signing and dating any consent form, volunteers will be allowed adequate time to consider the potential risks associated with their participation in the study.

A copy of all signed consent forms will be provided to the participant. Original signed and dated consent forms will be retained with the study records.

Only participants who sign the study-specific ICF are considered as enrolled in this trial.

19.5 Adherence to Protocol

The Investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6 (R3), PMRI SOPs, and all applicable guidelines and regulations.

19.6 Confidentiality

The collection, use, and disclosure of participant's personal information are detailed in the PCF which will be provided to the participants at the time of screening.

Pharma Medica Research Inc. and the Sponsor will preserve the confidentiality of all participants taking part in the study. If documents containing the participants' identifiable data are reproduced, the identifiable data will be omitted from the replica.

If results of this study are published, only numbers or symbols will be used to identify the participants. If photographs are required and published, the identities of the participants will be protected.

The Investigator and all employees and coworkers involved with this study must not disclose or use, for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to them for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties. Participants will have the right to access their personal health information in compliance with local legislation and regulations.

19.7 Study Reporting Requirements

By participating in this study, the Investigator agrees to submit reports of SAEs according to the timeline and method outlined in the protocol. In addition, the Investigator agrees to submit annual reports to the ERB/IRB as appropriate.

19.8 Financial Disclosure and Obligations

The Investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under local legislation and regulations. In addition, the Investigator must provide to the Sponsor any relevant changes that occur during the course of the study until one (1) year after the completion of the study.[12]

Neither the Sponsor nor PMRI is financially responsible for further testing or treatment of a participant for any medical condition that may be detected during the screening process.

19.9 Insurance

For the purpose of study related injury of participants, the Sponsor will have valid insurance for the duration of the study and for at least 30 days after the end of the study. PMRI will have valid liability insurance at all times participants are at the clinical facility.

20.0 Study Management

20.1 Protocol Revisions and Informed Consent Form Modifications

Changes or significant clarifications of the approved protocol have to be made in writing as a protocol revision.

Revisions to the protocol will be submitted to the ERB/IRB for approval.

Any revision that alters the information or procedures involving participants (i.e., written in the ICF) will require modification of the ICF. Modified ICFs will be reviewed and approved by the ERB/IRB prior to implementation. All active participants in each stage will be required to voluntarily consent to and sign the revised ICF as soon as possible.

20.2 Study Termination

Although the Sponsor has every intention of completing the study, they reserve the right to terminate the study at any time for clinical or administrative reasons.

The Investigator reserves the right to terminate the study at any time for safety reasons.

End of study will be the last scheduled procedure, or the date the last participant completes their last assessment, whichever is latter. Unscheduled visits and AE follow-up may occur after the end of scheduled study procedures.

20.3 Study Report

Whether the study is completed or prematurely terminated, PMRI will provide the Sponsor or designee with a report (i.e., full or summary) based on the Sponsor's requirements.

The reports will meet the standards of the ICH Harmonised Tripartite Guideline E3: Structure and Content of Clinical Study Reports.

All the study data included in the report will conform to the required standards specified in the FDA Data Standards Catalog.

20.4 Monitoring of the Study

20.4.1 On-Site Audits/Inspections

Representatives of the Sponsor may visit the clinical research facility to carry out an audit of the study in compliance with the regulatory guidelines and company policy. During the audit, the investigator will cooperate with the monitor to ensure that any discrepancies between source data and eCRF are resolved. The investigator must provide the auditor with direct access to all relevant documents and discuss any findings with the auditor. Such audits will require access to all study records, study drug(s), and source records (including but not limited to participant clinic records) for inspection and comparison. The Sponsor will provide sufficient notice to PMRI prior to the visit to adequately prepare for the audit.

Similar inspection procedures may also be conducted by agents of any regulatory body reviewing the results of this study in support of a Licensing Application. Pharma Medica Research Inc. will immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

20.4.2 Monitoring

The Sponsor and/or representative(s) of the Sponsor may visit the study site to check that the rights and well-being of clinical trial participants are protected, the quality of data is maintained and ensure that the investigative site is adhering to the study protocol. Additionally, the monitor will ensure that the site is following the legal and ethical requirements as stated in local laws and the principles of GCP.

During the visit, the monitor will review: study records, and directly compare them with source records (including, but not limited to subject hospital records), discuss the study conduct with the investigator and study staff, and verify that the investigator, study staff, and facilities remain acceptable for the conduct of the study. The Investigator will cooperate with the monitor to ensure that any discrepancies between source records and eCRFs are resolved. The staff of PMRI will be available to assist the Sponsor in answering any inquiries.

During these in-person monitoring visits, the participants' medical records, source records, and other study related documents, including drug accountability records and study drugs, will be made available for review.

20.4.3 Quality Assurance and Quality Control

Pharma Medica Research Inc. is responsible for maintaining Quality Assurance (QA) and Quality Control (QC) Systems with written SOPs to ensure the study is conducted and data is generated, documented, and reported in compliance with the protocol and the applicable regulatory requirements. These QA and QC SOPs will be followed during the conduct of this study. QC reviews include all study records; the scope of QA reviews are risk-based.

Internal audits will be conducted at pivotal times by PMRI QA and QC staff to ensure study integrity. A Quality Assurance Statement will be provided to the Sponsor following a QA audit.

20.5 Risk Management and Rescue Medication

Study Specific Risk Management/Mitigation Plan(s) and SOPs are in place and will be followed for the protection of participants and staff.

In case of a medical emergency, a crash cart with the necessary resuscitation medication and equipment, is available in the clinical facility.

20.6 Archives/Record Retention

Study related documents will be archived by PMRI, as required by the applicable regulatory requirements, including the current version of the GCP, for at least 15 years.[8] The Sponsor will be notified prior to the retention period completion.

21.0 References

1 Potvin, D, DiLiberti CE, Hauck WW, Parr AF, Schuirmann DJ, Smith RA. Sequential design approaches for bioequivalence studies with crossover design. *Pharmceut Statist*. 2007. DOI: 10.1002/pst.294

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