1 PROTOCOL SUMMARY

1.1 Synopsis

Title of study:	Open-label, randomized, 2-way crossover, monocentric, controlled study to evaluate the effect on daily PHE fluctuation of PKU GOLIKE versus standard of care in patients with phenylketonuria
Protocol number:	GLK-IT-2023 V3.0 – 13.02.2024
Sponsor:	Applied Pharma Research S.A.
Phase of development:	Post-market
Investigational site:	ASST Santi Paolo e Carlo, Presidio Ospedale San Paolo, Via Antonio di Rudinì 8 – 20142, Milano (Italy) – Clinical Department of Pediatrics.
Aim of the study	To document whether PKU Golike prolonged-release amino-acids formulation can achieve more stable phenylalanine blood levels with respect to the standard of care.
Study description:	This is an open-label, randomized, 2-way crossover, monocentric controlled study in patients (≥ 16 years old) with phenylketonuria (PKU). The comparison will be between the test product (PKU GOLIKE, a prolonged-release amino-acids (AAs) mixture) and standard of care. The study will consist of a screening visit (V_1), two treatment periods with two test days each and a final visit planned at the end of the second treatment period (V_2). Test days will occur on two consecutive preferably non-working/school days (T_1-T_2 and T_3-T_4,) preferably over two consecutive weeks. Treatment days in the two periods will have to be the same days of the week. T_1 should be within two weeks after V_1. The study products will be self-administered at home. Following informed consent and verification of eligibility criteria, 20 patients with PKU will be randomized in a 1:1 ratio to one of the following two sequences: AB or BA where A=standard of care and B=PKU GOLIKE. In details: - AB: patients will receive 3 daily self-administrations of their usual standard of care for 2 consecutive days in the first period followed by 3 daily self-administrations of PKU GOLIKE for 2 consecutive days in the second period. - BA: patients will receive 3 daily self-administrations of PKU GOLIKE for 2 consecutive days in the first period followed by 3 daily self-administrations of their usual standard of care for 2 consecutive days in the second period. No change in the randomization sequence will be allowed. Each patient will follow the same diet in terms of food, calories and nutrients ranges during the first days (T_1 and T_3) of each period and the same diet during the second days (T_2 and T_4) of each period, according to

<table>
<tr><td></td><td>the age and body weight of the patient. The days before the test days each patient will follow her/his standard diet.

PKU GOLIKE /standard of care will be the only protein substitute allowed on each test day, and no sports activities will be allowed on sampling days.

Five blood spots will be collected on the second test day of each period.

Timing of self-administrations, meals and blood spots will be standardized for each test day. On the test days, no food will be allowed outside of the defined time windows. The first self-administration of each test day will be performed after an overnight fasting (10 -12 h) and before any food intake.

A patient's e-diary will be used to collect information on patient compliance, 24-hour blood spot collections, diet, daily activities, adverse events and event-related concomitant medications.

Adverse events will be continuously monitored during the study, starting from informed consent. Adverse events will be collected by the patients (or by a parent/guardian) in the e-diary and during the telephone calls made by the Investigator to the patients. Moreover, patients (or parents/guardians) will be instructed to promptly report adverse events occurring during the study to the Investigator.

The end of study (V_2) will be performed (remotely, by phone) at the end of the second treatment period, within 2 weeks after the last test day. Patients prematurely discontinued from the study will be asked to attend (remotely, by phone) a discontinuation visit possibly taking place within 2 weeks from the last test day. At the final/discontinuation visit, the investigator will organize collection of blood spots test and residual PKU GOLIKE product from the patient's domicile through a dedicated courier service. The account of the e-diary will be inactivated.

Patients will receive a reimburse for the travel expenses, if required.</td></tr>
<tr><td>Objectives and endpoints:</td><td>
<table>
<tr><th>Objectives</th><th>Endpoints</th></tr>
<tr><td>Primary efficacy objective</td><td>Primary efficacy endpoint</td></tr>
<tr><td>To compare the impact of GOLIKE versus standard of care on phenylalanine (Phe) blood levels</td><td>Blood levels of Phe at pre-defined time points over 24 hours of each second test day.</td></tr>
<tr><td>Secondary efficacy objectives</td><td>Secondary efficacy endpoints</td></tr>
<tr><td>To compare the impact of GOLIKE versus standard of care on tyrosine (Tyr) blood levels.</td><td>Blood levels of Tyr at pre-defined time points over 24 hours of each second test day.</td></tr>
<tr><td>Exploratory objective</td><td>Exploratory endpoint</td></tr>
<tr><td>To compare the impact of GOLIKE versus standard of care on branched-chained amino acids (leucine, isoleucine, valine) blood levels.</td><td>Blood levels of branched-chained amino acids (leucine, isoleucine, valine) at pre-defined time points over 24 hours of each second test day.</td></tr>
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<tr><td>Study population:</td><td>The study will enroll patients ≥16 years of age with PKU diagnosed at newborn screening and under a low Phe diet since diagnosis.

Inclusion Criteria

1. Signed, informed consent obtained by the patient prior to being enrolled into the study and prior to starting any data collection. For</td></tr>
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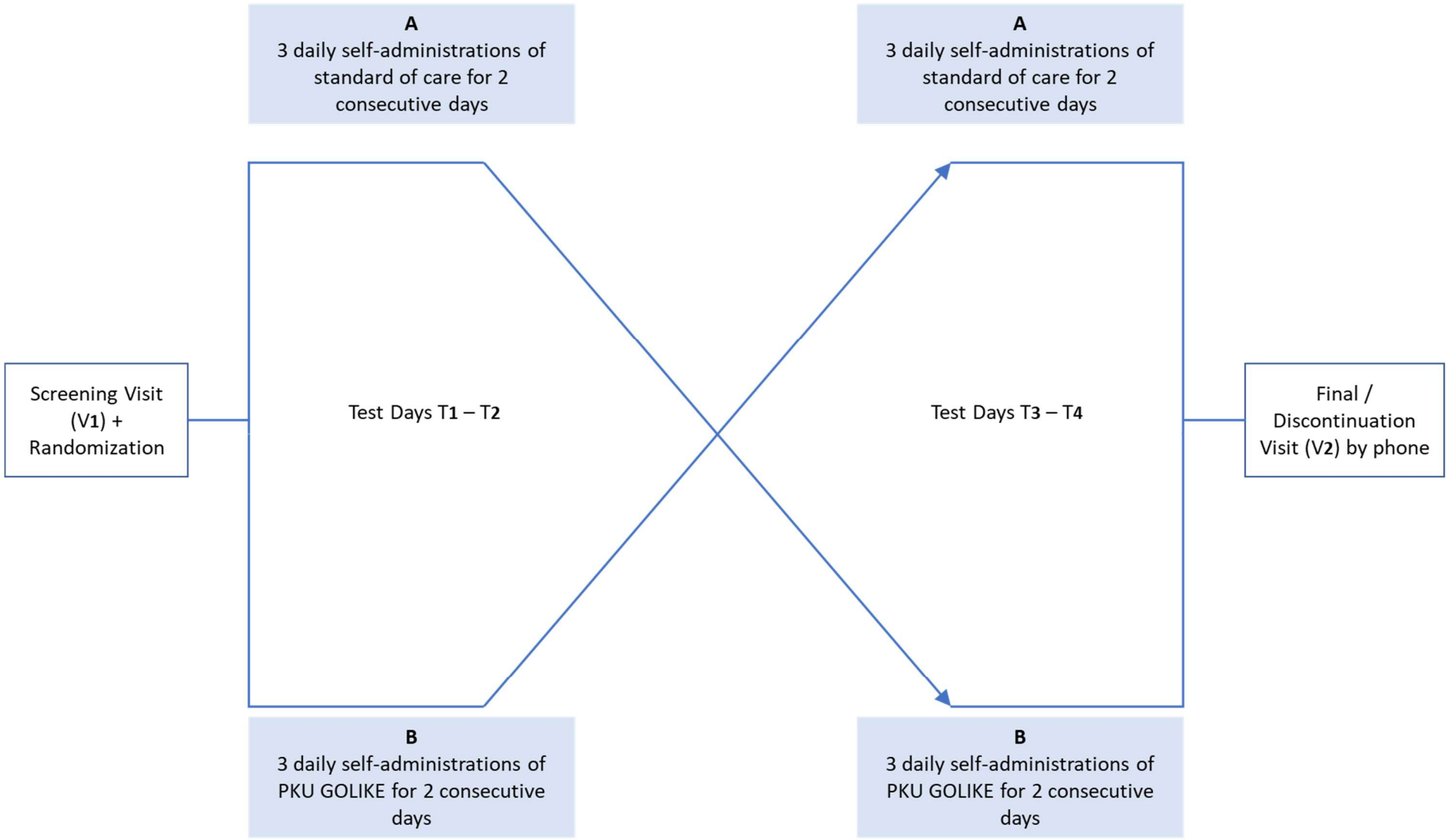
	legally minor patients, signed written consent shall be obtained also by the parents/legal guardian. 2. Male or female, aged ≥16 years. 3. Patients with a registered diagnosis of PKU 4. Ability and willingness to comply with all study procedures and availability for the duration of the study. 5. Patients with mean value of blood Phe >360 µM in the previous 12 months (calculated on at least 3 samples during the previous 12 months; the last sample should be preferably obtained in the 30 days preceding inclusion in the study). 6. Patient taking free-AA and/or GMP as usual amino-acids supplementation. **Exclusion Criteria** 1. Known or suspected hypersensitivity to any excipients/components of PKU GOLIKE. 2. Treatment with any drug therapy for PKU 3. Patient taking PKU GOLIKE as usual amino-acids supplementation 4. Patient taking LNAA as usual amino-acids supplementation 5. Any moderate to severe medical condition, which in the opinion of the Investigator would interfere with the study procedures or study outcome (reason to be provided) 6. Any current participation in another clinical trial involving investigational or marketed products in the 3 months prior to the inclusion in this study. 7. Pregnancy or lactation.
Study intervention:	**Test product:** PKU GOLIKE, a prolonged-release amino-acids (AAs) mixture. PKU GOLIKE is a FSMP for the dietary management of PKU. The product will be supplied in two formulations: PKU GOLIKE PLUS 16+ (granules) and PKU GOLIKE KRUNCH (tablets). For the test product, dosing will be adjusted by body weight in order to provide a PE total daily dose equal to the one in use by each patient in his standard of care (X g/kg). The prescribed formulation will be the granules but, at discretion of the physician, a part of the dose administered with the lunch on the first test day can be replaced with the tablets. **Reference product:** standard of care Test and reference products will be self-administered by patients at home and will be taken with food or liquid, according to the product leaflet.
Timing of self-administrations, meals and blood spots:	<u>First test day with 3 self-administrations of either standard of care or PKU GOLIKE:</u> – h. 8:00: 1st product self-administration with breakfast – h 12:00: 2nd product self-administration with lunch – h 16:00: afternoon snack – h 20:00: 3rd product self-administration with dinner <u>Second test days with 3 self-administrations of either standard of care or PKU GOLIKE:</u> – h 8:00 (±15 min): 1st blood spot

	– 1st product self-administration (right after the blood spot) with breakfast – h 12:00 (±15 min): 2nd blood spot – 2nd product self-administration (right after the blood spot) with lunch – h 16:00 (±15 min): 3rd blood spot – right after the blood spot afternoon snack – h 20:00 (±15 min): 4th blood spot – 3rd product self-administration (right after the blood spot) with dinner – h 8:00 (±15 min) (following morning): 5th blood spot (in fasting conditions, before breakfast) On the test days, no food will be allowed outside of the defined time windows.
Sample size determination:	Considering the nature of the study [crossover, randomized, controlled with standard care (SC), efficacy of PKU GOLIKE (PG) in reducing the variation of Phe in comparison to SC], the testing hypothesis is to observe a major effect of the PG versus SC. 20 patients are adequate to demonstrate a difference of 30 micromoles/L with 40 micromoles/L of standard deviation between treatments at any blood spots with 80% power and 5% for two-sided test. Patients that are not compliant or need to be excluded for other clinical reasons from the treatment schedule will be replaced in order to ensure 20 subjects completing both treatment sequences.
Statistical methods:	<u>General statistical methods</u> Descriptive statistics (mean with standard deviation, minimum and maximum, median with the first and third quartile and 95% confidence interval) will be calculated for quantitative variables. For qualitative variables, counts and percentages will be provided together with the 95% confidence interval. In calculation of percentages, patients with missing data will not be considered, unless otherwise specified. <u>Analysis of the primary and secondary efficacy endpoints:</u> The primary endpoint will be the comparison of the Phe at each time point by means of ANOVA for repeated measure (Wallenstein design) and post-hoc Dunnett's t test for multiple comparisons. The secondary endpoints time profile of Tyr, Leu, isoLeu and Val will be evaluated in blood spots with ANOVA for repeated measure (Wallenstein) and tested at each time points with post-hoc Dunnett's t.
Duration of study:	Patients will be followed until the final visit/discontinuation visit, which will take place at the latest within 2 weeks from the last test day.
Protocol version/date:	V 3.0 dated Feb 13, 2024

1.2 Study Design



1.3 Schedule of Events (SoE)

		1st period		2nd period		
Study period	**Screening Visit (V_1)**	**Test Day 1 (T_1)**	**Test Day 2 (T_2)**	**Test Day 3 (T_3) ***	**Test Day 4 (T_4)**	**Final / Discontinuation Visit (V_2) §**
Informed Consent signature	■					
Inclusion/Exclusion Criteria	■					
Demographics [1]	■					
Disease history [2]	■					
Relevant Medical history & Prior medications	■					
Anthropometric measures [3]	■					
Urine pregnancy test (if applicable)	■					
Diet preferences & Nutritional counseling [4]	■					
Information on dosage/way of administration of SoC	■					
GOLIKE PKU dispensing	■					
Randomization [5]	■					
Product self-administration according to randomization sequence [6]		■	■	■	■	
Blood spots [7]			■		■	
Blood spots collection by designated courier [8]						■
Adverse events [9]		Continuously through a patient's diary				■
Concomitant medications [10]	■	Continuously through a patient's diary				■
Diet [11]		On each test day through a patient's diary				■

Daily activities [12]		On each test day through a patient's diary	■
Compliance [13]		On each test day through a patient's diary	■
Information on 24-hour blood spots collection [14]		On each test day through a patient's diary	■

* Test days will occur on two consecutive preferably non-working/school days (T_1-T_2 and T_3-T_4) preferably over two consecutive weeks. Treatment days in the two periods will have to be the same days of the week. T_1 should be within two weeks after V_1. The second period is preferably to be done the week after the first period; nevertheless, one week of delay is admitted.

§ The end of study will be performed (remotely, by phone) at the end of the second treatment period, within 2 weeks from the last test day. Patients prematurely discontinued from the study will be asked to attend (remotely, by phone) a discontinuation visit possibly taking place within 2 weeks from the last test day.

[1] Demographics information (age and sex).

[2] Disease history (year of phenylketonuria (PKU) diagnosis, date of start of low phenylalanine (Phe) diet, prior/concomitant treatments/diet for PKU in the last 3 months, disease severity according to classification described in the article at https://doi.org/10.1002/edm2.396; current standard of care to be documented).

[3] Anthropometric parameters (weight, height, and body mass index (BMI)).

[4] Information on recommended dietary patterns (food, calories and nutrient content) and diet preferences will be collected. Moreover, each patient will receive nutritional counselling for diet standardization to be followed on each test day. Diet instructions will be provided to the patient.

[5] Randomization in a 1:1 ratio to one of the following two randomization sequences: AB or BA where A=standard of care and B=PKU GOLIKE. [6] Products will be self-administered by the patients at home. For each period, patients will receive 3 daily self-administrations for 2 consecutive days in accordance to the assigned randomized sequence. The first self-administration of each test day will be performed after an overnight fasting (10-12 h) and before any food intake.

[7] Five blood spots to be collected by the patients on the second test day of each period at home, following the instructions received by the Investigator. Blood spots will be collected at 8:00 (±15 min) right before product self-administration and before any food intake, at 12:00 (±15 min) right before product self-administration and before food intake, at 16:00 (±15 min) before food intake, at 20:00 (±15 min) right before product self-administration and before food intake, and at 8:00 (±15 min) on the following morning, before any food intake.

[8] At the final/discontinuation visit, the investigator will organize collection of blood spots test at the patient's home by a dedicated courier.

[9] Patients (or parent(s)/guardian) will be instructed by the Investigator to record any signs, symptoms or medical events occurring during the study until the final/discontinuation visit on a patient's diary. The participant (or parent(s)/guardian) will be instructed to record the events starting on the day they happened and indicate in the diary when the events ended. Moreover, patients (or parent(s)/guardian) will be instructed to report adverse events timely

to the Investigator. All adverse events (AEs) are to be reported by the Investigator starting from he informed consent until 30 days after the final dose of the study product. After this period, only serious AEs suspected of being related to the study product are to be reported.

[10] Any new (not already reported in the medical history as a current therapy) concomitant medication (including any supplements) taken during the study will be recorded on a patient's diary.

[11]Each patient will follow the same diet (= food ingested over 24 hours) in terms of food, calories and nutrients ranges during the first days (T_1 and T_3) of each period and the same diet during the second days (T_2 and T_4) of each period, according to the age and body weight of the patient. The days before the test days each patient will follow his standard diet. On the test days, no food will be allowed outside of the defined time windows.

[12] Information on the daily activities of each patient will be collected in the patient's diary. No sport activities are allowed on sampling days

[13] Information on compliance to the study product will be collected in the patient's diary on each test day. This information will include but will not be limited to information on the amount of product weighed and on whether the suspension/solution is ingested.

[14] Information on 24-hour blood spots will be collected in the patient's diary on the second test day of each period. This information will include but will not be limited to the time of sample collection and information on whether some blood spots are not collected.

1.4 Details of test days

	First test days				Second test days								
	h 8:00	**h 12:00**	**h 16:00**	**h 20:00**	**h 8:00 (±15 min)**	**Right after the blood spot**	**h 12:00 (±15 min)**	**Right after the blood spot**	**h 16:00 (±15 min)**	**Right after the third blood spot**	**h 20:00 (±15 min)**	**Right after the blood spot**	**h 8:00 (±15 min)**
Blood spots *					■		■		■		■		■
Product self-administration	■	■		■		■		■				■	
Meal §	■	■	■	■		■		■		■		■	
Reporting of information in the patient's diary #	Following the Instructions received by the Investigator												

* Blood spots must be collected before product self-administration and before food intake.

§ No food is allowed outside of the defined time windows.

Information on patient's compliance, 24-hour blood spots collection, diet, daily activities, adverse events, and concomitant medications.