CLINICAL STUDY SYNB1934-CP-003

Protocol Version 4.0_US: 01 July 2023

Study Title:	A Phase 3, Double-blind, Placebo-controlled, Randomized Withdrawal Study to Evaluate the Efficacy and Safety of SYNB1934 in Patients with PKU (SYNPHENY-3)
Study No.:	SYNB1934-CP-003
Short Title:	Synpheny-3
Study Phase:	2b/3
Product Name:	SYNB1934v1
IND No.	IND 27340
EU Trial No.	2022-502932-37-00
HC Control No.	273002
Indication:	Phenylketonuria
Sponsor:	Synlogic Operating Company, Inc. 301 Binney Street, Suite 402 Cambridge, MA 02142 USA Phone: +1 (617) 401-9975

Revision History		
Version	**Date**	**Reason for Change**
1.0	06 December 2022	Original version
2.0_CA 2.0_EU 2.0_IL 2.0_TR 2.0_GE	21 February 2023; Canada 21February2023; European Union 21 February 2023; Israel 21 February 2023; Turkey 21 February 2023; Georgia	• Addition of a minimum number of participants 12-17. • More complete description of dose ramping in OLE. • Clarification of statistical measures. • Removal of optional TCA substudy. • Simplification of pregnancy testing
3.0_US	08 May 2023, United States	Description of dose ramping in OLE, clarification of statistical measures, removal of TCA substudy. Response to regulatory guidance: addition of weekly Phe substudy and alteration of inclusion age. [Note: Version 2.0 was not released in the US; Version 3.0 captures all changes made in Version 2.0]
4.0_US	01 July 2023, United States	Timing of diary card collection, alteration of AESI criteria, clarification of responder population.

SIGNATURE PAGE (SPONSOR)

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1934-CP-003 Version 4.0_US, dated 01 July 2023, and agree to meet all obligations of Synlogic Operating Company, Inc., as detailed in all applicable regulations and guidelines. In addition, I will ensure that the lead investigators are informed of all relevant information that becomes available during the conduct of the study.

[redacted]

Synlogic Operating Company, Inc.

_____

Date

LEAD INVESTIGATOR'S AGREEMENT

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1934-CP-003 Version 4.0_US, dated 01 July 2023, and will adhere to the study requirements as presented, including all statements regarding confidentiality. In addition, I will conduct the study in accordance with the requirements of this protocol and also protect the rights, safety, privacy and well-being of study participants in accordance with the following:

- International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) E6(R1).
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Requirements for reporting serious adverse events as defined in Section 6 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.

My signature also acknowledges that:

- Neither my subinvestigators nor I are members of the Institutional Review Board reviewing this protocol, or
- I and/or my subinvestigators are members of the Institutional Review Board, but I/we will not participate in the initial review or continuing review of this study.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Name of Sponsor/Company: Synlogic Operating Company, Inc.

Name of Investigational Product: SYNB1934v1

Title of Study: A Phase 3, Double-blind, Placebo-controlled, Randomized Withdrawal Study to Evaluate the Efficacy and Safety of SYNB1934 in Patients with PKU (SYNPHENY-3)

Protocol Number: SYNB1934-CP-003

Phase of Development: 2b/3

Objectives and Endpoints

This study has 3 parts, and each study part has a distinct set of objectives, endpoints, and analysis populations. The primary determination of efficacy will be based on the comparison of SYNB1934v1 versus placebo in the randomized withdrawal period (Part 2).

Dose Escalating, Open-Label Period (DEP; Part 1):

Objective	Endpoint
Primary:	
• To assess the percentage change in blood phenylalanine (Phe) level	• Percent change from baseline in blood Phe level at last measurement of the individually titrated dose (iTD) of SYNB1934v1
Key Secondary:	
• To assess the absolute change in Phe level	• Change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1
• To determine the responder population	• A ≥ 20% reduction from baseline in blood Phe level at any time in the DEP
Other Secondary:	
• To assess proportion of participants achieving blood Phe level ≤ 360 µmol/L	• Blood Phe level ≤ 360 µmol/L at any time of the iTD of SYNB1934v1 • Blood Phe level ≤ 360 µmol/L at any time in the DEP
• To assess the safety and tolerability of SYNB1934v1	• Incidence and severity of adverse events • Changes from baseline in clinical laboratory parameters • Changes from baseline in vital signs measurements
Exploratory:	
[redacted]	[redacted]
[redacted]	[redacted]

• [redacted]	[redacted]
[redacted]	[redacted]

Note: baseline and week numbering are specific to study Part 1, unless explicitly noted otherwise in the endpoint.

Randomized Withdrawal Period (RWP; Part 2):

Objective	Endpoint
Primary:	
• To evaluate efficacy of SYNB1934v1 versus placebo in the responder population	• Change from baseline to Week 4 in blood Phe level
Key Secondary:	
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to percent change from DEP baseline in blood Phe level	• Percent change from DEP baseline in blood Phe level at Week 4
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to change from DEP baseline in blood Phe level	• Change from DEP baseline to Week 4 in blood Phe level
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to the proportion of participants with a blood Phe level ≤ 360 µmol/L	• Blood Phe level ≤ 360 µmol/L at Week 4
Other Secondary Objectives:	
• To assess the safety and tolerability of SYNB1934v1	• Incidence and severity of adverse events • Changes from baseline in clinical laboratory parameters • Change from baseline in vital signs measurements
• To evaluate efficacy of SYNB1934v1 versus placebo in the responder population with regard to the proportion of participants with a ≥ 20% reduction from DEP baseline in blood Phe level at Week 4	• A ≥ 20% reduction from DEP baseline in blood Phe level at Week 4
Exploratory:	
• [redacted]	[redacted]

• [redacted]	[redacted]
• [redacted]	• [redacted]

Note: baseline and week numbering are specific to study Part 2, unless explicitly noted otherwise in the endpoint.

Open-Label Extension (OLE; Part 3):

Objective	Endpoint
• [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Note: baseline and week numbering are specific to study Part 3, unless explicitly noted otherwise in the endpoint.

Methodology

This is a 3-part, adaptive study consisting of a DEP of up to 15 weeks (Part 1), followed by a 4-week, double-blind, placebo-controlled RWP (Part 2), and an OLE (Part 3) of up to 36 months.

DEP (Part 1):

In the DEP, all enrolled participants will maintain a stable diet reflecting their baseline Phe intake and receive escalating doses of SYNB1934v1 from approximately 3 to 15 weeks to determine an iTD, which is defined as the highest dose the participant is able to tolerate. A participant will be defined as having reached an iTD if they tolerate 3 weeks at a dose, regardless of whether other doses are tolerated.

If a participant tolerates escalation to the initial dose (Dose 1) after 3 weeks, the investigator will escalate to Dose 2. If Dose 2 is tolerated, the participant will continue on this dose for a total duration of 3 weeks prior to attempting to escalate to Dose 3. A participant will stay on his or her iTD for 3 weeks. If at any time during a 3-week dosing interval the participant or investigator considers a dose level to be intolerable, the dose may be de-escalated after discussion with the medical monitor. In this case, the participant will restart 3 weeks at the lower level iTD. After de-escalation, participants cannot escalate to a higher dose again during Part 1. In general, a dose is considered intolerable if the participant experiences Grade 3 or more severe adverse events (AEs) or Grade 2 gastrointestinal (GI) AEs that do not improve with continued dosing at the same dose level over time. If a dose is de-escalated due to a reason other than Grade 3 or greater, or Grade 2 GI AEs, or a second de-escalation is required, the medical monitor must be consulted.

Blood Phe level will be measured at each dose level after 3 weeks at that level. A responder will be defined as a participant who achieves a ≥ 20% reduction in blood Phe level compared to DEP baseline on SYNB1934v1.

The DEP will contain an adaptive, seamless Phase 2b component. Once the first 20 participants have attained an iTD and completed the DEP or the 30th participant has a baseline DEP and has been in the study for 105 days, DEP data will be evaluated by an independent data monitoring committee (DMC) using a pre-specified set of criteria based on both safety and efficacy to determine whether the dose regimen is appropriate or whether any doses should be discontinued as well as whether the study should be stopped for futility.

[redacted]

RWP (Part 2):

All participants who complete the DEP at their iTD for at least 3 weeks will enter a 4-week RWP. Participants will be randomized 1:1 to receive SYNB1934v1 at their iTD determined in the DEP or placebo 3 times daily (TID). Randomization will be stratified on screening Phe level. Participants will remain on the assigned dose (iTD of SYNB1934v1 or matching placebo) for the duration of the RWP, unless they develop intolerance or meet other discontinuation criteria, and will remain on the same diet they consumed during the DEP. Blood Phe level will be measured at Week 1, 3 and 4 of the RWP.

OLE (Part 3):

Participants who complete the 4-week RWP may enter the OLE and receive SYNB1934v1 for up to 36 months. (At the sponsor's discretion, enrollment directly into OLE may be opened.) During the OLE all participants will complete a dose ramp to their iTD over time guided by tolerability. The iTD in OLE may be different from the iTD in DEP. The investigator may escalate the dose of SYNB1934v1 up to a maximum of 1×10^{12} live cells based on tolerability; multiple attempts to escalate to a higher dose level are permitted per investigator discretion. Participants will be allowed to modify their standard diet if their blood Phe level is < 240 µmol/L, with guidance from the investigator

as outlined in the study-specific Diet Manual. The 3-day dietary intake assessments will continue per the Schedule of Events.

Dosing and Dose Escalation

SYNB1934v1 will be taken with a meal throughout the study. In the DEP, participants will complete a dose ramp at the beginning of each dose level.

The escalating dose levels are as follows:

- Dose 1: 3×10^{11} live cells
- Dose 2: 6×10^{11} live cells
- Dose 3: 1×10^{12} live cells

During participation in the study, participants will take a proton pump inhibitor (PPI; esomeprazole 20 mg or equivalent) once per day (QD; recommended before breakfast), starting 7 days before the first dose of SYNB1934v1 in Part 1. If they cannot tolerate a PPI, they can use an H2 blocker per investigator discretion. If neither the PPI nor H2 blocker can be tolerated, the participant will discontinue from the study. The dose of PPI or H2 blocker should remain stable during the DEP and RWP; if a dose adjustment to the PPI or H2 blocker is required in OLE, the investigator must discuss with the medical monitor (unless the adjustment is required due to an adverse event).

Diet and Phe Monitoring

For 3 consecutive days prior to scheduled study visits and blood Phe draws, all participants will have a 3-day dietary intake assessment. A study dietitian will interview the participant and provide advice on maintaining a diet that is consistent with baseline diet throughout the study. Participants will follow their usual diet (including Phe, protein, and medical food intake) from baseline in the DEP to the end of the RWP.

During the DEP and RWP, blood Phe and tyrosine (Tyr) levels will be blinded to the sponsor, participant, and investigator. The investigator will be notified if Phe is > 1.5 × the DEP baseline value, if Phe falls below 30 µmol/L or if Tyr falls below 20 µmol/L to allow for individual safety discontinuation. During the OLE, participants will be allowed to modify their standard diet if their blood Phe level is < 240 µmol/L, with guidance from the investigator as outlined in the study-specific Diet Manual. The 3-day dietary intake assessments will continue per the Schedule of Events.

Number of Participants (planned): [redacted] are planned to be enrolled.

Study Inclusion and Exclusion Criteria

Male and female participants will be enrolled.

Inclusion Criteria:

1. Age ≥ 18 years.
2. Able and willing to voluntarily complete the informed consent process
3. Diagnosis of phenylketonuria (PKU) and failure to maintain recommended blood Phe levels on existing management (sapropterin, sepiapterin and/or Phe-restricted diet), demonstrated by uncontrolled blood Phe level > 360 µmol/L on current therapy any time during screening and uncontrolled blood Phe level > 360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the participant's medical history (inclusive of any screening values). All screening values must be obtained more than 7 days apart, as determined by central or local laboratory.

4. Females of childbearing potential must have a negative pregnancy test at screening and the end of DEP (in order to enter the RWP) and RWP (in order to enter the OLE) and be willing to have additional pregnancy tests during the study.
5. Sexually active female participants of childbearing potential must be willing to use an acceptable method of contraception while participating in the study and for 2 weeks after the last dose.
6. Stable diet including stable medical formula regimen (if used) for at least 1 month prior to screening.
7. If using sapropterin or sepiapterin, must be on a stable dose for at least 3 months.
8. Willing and able to continue current diet, sapropterin, sepiapterin and large neutral amino acids unchanged during screening, DEP and RWP and to engage in all study activities.

Exclusion Criteria:

1. Currently taking Palynziq® (pegvaliase-pqpz) (within 1 month of screening).
2. Acute or chronic medical, surgical, psychiatric, or social condition or laboratory abnormality that may increase participant risk associated with study participation, compromise adherence to study procedures and requirements, and, in the judgment of the investigator, would make the participant inappropriate for enrollment.
3. A known or suspected diagnosis of DNAJC12 deficiency, biopterin synthesis deficiency, or irritable bowel syndrome.
4. Intolerance to or allergic reaction to *E. coli* Nissle or any of the ingredients in SYNB1934v1 formulation, or an allergy to cinnamon. Known intolerance to proton pump inhibitors and H2 blockers, since one or the other must be used.
5. Currently taking or plans to take any type of systemic (e.g., oral or intravenous) antibiotic within 28 days prior to the first dose of SYNB1934v1 through final safety assessment in RWP, including planned surgery, hospitalizations, dental procedures, or interventional studies that are expected to require antibiotics. Exception: topical antibiotics are allowed.
6. Pregnant, planning to become pregnant, or breastfeeding.
7. Current participation in any other investigational drug study or use of any investigational agent within 30 days or 5 half-lives (whichever is longer) prior to screening.
8. Ever received gene therapy for treatment of PKU.

Study Drug: SYNB1934v1 at 3×10^{11}, 6×10^{11}, or 1×10^{12} live cells or matching placebo

Duration of Treatment

- Screening period: up to 45 days
- Part 1: DEP: up to 15 weeks
- Part 2: RWP: 4 weeks
- Part 3: OLE: up to 36 months

Reference Therapy, Dosage, and Mode of Administration: Placebo to match SYNB1934v1

Removal of Individual Participants from Investigational Medicinal Product and Withdrawal of Consent

Reasons for discontinuation of investigational medicinal product (IMP; i.e., SYNB1934v1 or placebo) may include the following:

- The participant withdraws consent.
- The investigator or sponsor notes a significant noncompliance with protocol procedures.
- The participant develops an intolerable toxicity including but not limited to a Grade 3 or more severe adverse event or serious adverse event (SAE) assessed as related to IMP by the investigator.
- The investigator determines that the participant must discontinue further study dosing for medical reasons (including pregnancy).
- The participant has low levels of Phe (< 30 µmol/L) or Tyr (< 20 µmol/L) or has profound elevations in Phe (> 1.5 × the DEP baseline value during DEP and RWP and > 2200µM during OLE).

If possible, the investigator will confer with the sponsor or medical monitor before discontinuing dosing. Participants who discontinue from IMP in RWP will be encouraged to remain on study and complete assessments (particularly safety assessments) through the remainder of RWP of the study. Participants who discontinue from DEP and OLE will be asked to have an early termination visit 30 days after the last dose of IMP.

Participants may withdraw their consent at any time for any reason without prejudice to their future medical care by the physician or at the institution. If a participant withdraws consent, the date and stated reason for consent withdrawal should be documented. Participant data collected up to the date of consent withdrawal will be included in the analyses.

Study Stopping Rules

If any of the following criteria are met, enrollment will be halted. Participants already enrolled in the study will continue dosing until review by the Data Monitoring Committee (DMC).

1. Two or more participants experience an AE ≥ Grade 3, per protocol severity grading criteria, related to SYNB1934v1 as assessed by the investigator.
2. Clinical infection is detected in a sterile space with a gram-negative aerobic bacillus confirmed by qPCR to be SYNB1934v1.

If any of the above criteria are met, the DMC will review all relevant data and determine whether enrollment in the study should resume and whether any modifications to the study are warranted. If the DMC decides that further enrollment should be permanently suspended, subjects already enrolled in the study will continue to be followed for the duration of the study.

If a death occurs at any time during the study that is considered by the investigator to be related to SYNB1934v1, enrollment will be halted and dosing for subjects already enrolled will be stopped until the DMC reviews all relevant data and makes further recommendations.

Statistical Methods

General:

All evaluations and tabulations will be carried out as described in detail in a statistical analysis plan (SAP), which will be finalized and approved prior to the DEP interim analysis and database lock and unblinding.

A disposition of participants will be provided for each of the 3 parts of the study.

Continuous variables will be summarized using the number of observations, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages.

Baseline for blood Phe level in each of the 3 parts of the study will be defined as the mean of the duplicate blood Phe level measurements obtained immediately prior to administration of the first dose of each part. If only one blood Phe level measurement is available, then that measure will be used as baseline. Similarly, for the determination of responders and participants who achieve a specific reduction in Phe (e.g., Phe level ≤ 360 μmol/L at any time in the DEP, or a ≥ 20% reduction from baseline) the mean of the duplicate blood Phe level measurements at the applicable post-baseline timepoint (e.g., Week 3) will be used. If only one of the duplicate blood Phe levels is available at a particular post-baseline timepoint, then that measurement will be used.

In describing the endpoints, the baseline and week numbering are specific to the corresponding study part, unless explicitly noted otherwise.

Analysis Populations:

Analysis populations will be defined for each of the 3 parts of the study (DEP, RWP, and OLE).

Full analysis set (FAS): The full analysis sets are based on the intention-to-treat principle. The DEP FAS includes all participants enrolled in the DEP.

The RWP FAS is defined as all participants randomized into the RWP. Additionally:

- The responder RWP FAS is defined as participants in the RWP FAS who are responders
- The nonresponder RWP FAS is defined as participants in the RWP FAS who are nonresponders

A responder is defined as a participant who achieves a ≥ 20% reduction in blood Phe level compared to DEP baseline on SYNB1934v1.

The responder RWP FAS will be the primary efficacy analysis population for the RWP. Analyses on the RWP FAS will be performed according to the randomized treatment group.

In the event that, based on the interim analysis results utilizing the protocol-defined DMC criteria for dose changes and futility, the DMC recommends dropping either the 1×10^{12} or the 1×10^{12} and 6×10^{11} doses, the DEP FAS will exclude all participants who were enrolled and treated at a dropped dose in the DEP. Additionally, the RWP FAS will exclude all participants who have been randomized in the RWP up to this time. The participants in the DEP who have an iTD corresponding to a dropped dose will go directly into the OLE part of the study (i.e., as opposed to being randomized into the RWP).

These participants who are excluded from the DEP FAS and RWP FAS will be summarized separately.

Safety analysis set (SAS): For each of the 3 study parts, the SAS will include all participants who received any amount of IMP in the corresponding study part with treatment assignment based on the treatment received.

Per-protocol analysis set (PPS): The DEP PPS is defined as all DEP FAS participants, who had no major protocol deviations in DEP that would affect efficacy, completed their final 3-week iTD dosing period, whose dietary Phe remained within 20% of their DEP baseline value, and who did not require systemic antibiotic treatment during DEP. The DEP PPS will be used as a sensitivity analysis for the DEP primary efficacy endpoint.

The responder RWP PPS is defined as all responder RWP FAS participants, who had no major protocol deviations that would affect efficacy, had at least 75% treatment compliance, whose dietary Phe remained within 20% of their DEP baseline value, and who did not require systemic antibiotic treatment during DEP or RWP. The responder RWP PPS will be used as a sensitivity analysis for the RWP primary efficacy endpoint. Major protocol deviations will be determined prior to the RWP database lock. Analyses on the responder RWP PPS will be performed according to the randomized treatment group.

Sample Size Considerations:

[redacted]. This assumes a dropout rate of 10% in the DEP and approximately 50% of participants are responders. If, during the course of the study, it becomes apparent that the assumptions were not accurate or a dose level is dropped after the interim analysis, the number of participants enrolled into the DEP may be adjusted. The study has over 98% power in testing that the mean percent change from baseline at the last measurement of the iTD in blood Phe level is different from 0. This assumes a mean percent reduction in blood Phe levels of -11%, a standard deviation of 17%, a 10% drop-out rate, and a 2-sided Type I error rate of 0.05. The mean percent reduction and standard deviation in blood Phe values used in the power calculations are based on results from a similar study in participants with PKU.

A sample size of [redacted] in the responder RWP [redacted] to detect a difference in the mean change in blood Phe level from baseline to Week 4 between the SYNB1934v1 and placebo treatment groups. This assumes a mean treatment group difference in blood Phe of -245 µmol/L between the SYNB1934v1 and placebo groups, a change from baseline standard deviation of 247 µmol/L, 1:1 randomization of SYNB1934v1 and placebo, a 5% drop-out rate in the RWP, and a 2-sided Type I error rate of 0.05. The power was calculated via a t-test. The mean treatment group difference and standard deviation in blood Phe values used in the power calculations are based on results from a similar study in participants with PKU.

Primary Efficacy Analysis:

DEP (Part 1): The DEP primary efficacy endpoint is the percent change from DEP baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1. The last measurement is the participant's last Week 3 blood Phe level at the iTD of SYNB1934v1.

The mean percent change from baseline at the participant's iTD will be tested by a mixed-model with repeated measures (MMRM) analysis. The MMRM model will have DEP baseline blood Phe level and iTD dose level as fixed effects. The dependent variable is the percent change from baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1.

Least-squares means for the percent change from baseline overall and at each iTD dose level, along with the corresponding [redacted] and p-values testing a percent change from baseline equal to 0, will be calculated. The primary efficacy analysis will test the least-squares means percent change from baseline equal to 0 for the blood Phe level at the participants' last week (Week 3) at his or her established iTD.

At each of the scheduled assessments blood Phe will be drawn in duplicate. The MMRM will incorporate these participant repeated measures. For the MMRM analysis, the results of the post-baseline duplicate samples from the Week 3 visit will be included in the statistical model (i.e., without averaging the results within a given visit before inclusion into the MMRM).

The DEP FAS will be the primary efficacy analysis population.

RWP (Part 2): The RWP primary efficacy endpoint is the change from RWP baseline to Week 4 in blood Phe level. An MMRM analysis will be used to compare the mean change in blood Phe level between the placebo and SYNB1934v1 dose groups. The MMRM model will have treatment group,

RWP baseline blood Phe level, iTD dose level, visit, and visit × treatment group (interaction effect), visit × iTD dose level (interaction effect), and visit × RWP baseline blood Phe level (interaction effect) as fixed effects. The MMRM will incorporate the participant's repeated measures at visit Weeks 1, 3, and 4, with visit being treated as a categorical variable. Least-squares means for each treatment group and the SYNB1934v1 treatment group difference from placebo, along with the corresponding [redacted] and p-values will be calculated for Weeks 1, 3, and 4. The primary efficacy analysis will compare the least-squares means treatment difference between SYNB1934v1 and placebo in the change from baseline in the blood Phe level at Week 4.

At each of the scheduled assessments, blood Phe will be drawn in duplicate. The MMRM will incorporate these participant repeated measures. For the MMRM analysis, the results of each of the post-baseline duplicate samples from a visit will be included in the statistical model (i.e, without averaging the results within a given visit before inclusion into the MMRM). The responder RWP FAS will be the primary efficacy analysis population.

Key Secondary Efficacy Endpoint Analyses:

DEP (Part 1): The key secondary efficacy endpoints for the DEP are listed in the objectives and endpoints table above. The key secondary endpoint, change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1, will be analyzed using an MMRM analysis similar to the model described for the DEP primary efficacy analysis, with the dependent variable being the change in blood Phe level from DEP baseline.

For the secondary endpoint, a ≥ 20% reduction from baseline in blood Phe level at any time in the DEP, the proportion of participants meeting these criteria will be summarized along with 95% [redacted]

These analyses will be performed on the DEP FAS.

RWP (Part 2): The key secondary efficacy endpoints for the RWP are listed in the objectives and endpoints table above. The proportion of participants meeting the key secondary endpoint, a blood Phe level ≤ 360 µmol/L at Week 4, will be tested between the placebo and SYNB1934v1 dose groups using a Fisher's Exact test. The key secondary endpoint, change from DEP baseline to Week 4 in blood Phe level, will be analyzed using an MMRM analysis similar to the model described for the RWP primary efficacy analysis, with the dependent variable being the change in blood Phe level from DEP baseline, and the DEP baseline blood Phe level as the baseline blood Phe level covariate. The key secondary endpoint, percent change from DEP baseline in blood Phe level at Week 4, will be analyzed using an MMRM analysis similar to the model described for the RWP primary efficacy analysis, with the dependent variable being the percent change in blood Phe level from DEP baseline, and the DEP baseline blood Phe level as the baseline blood Phe level covariate.

These analyses will be performed on the responder RWP FAS.

Analyses of other secondary endpoints are described in the full protocol and will be included in the SAP. Analyses of exploratory endpoints will be described in the SAP.

Interim Analysis:

Once the first [redacted] have attained an iTD and completed the DEP or the [redacted] participant has a baseline DEP and has been in the study for [redacted], whichever occurs earlier, the DEP data will be evaluated by an independent DMC. The DMC will use the below pre-specified set of criteria based on both safety and efficacy to determine whether the dose regimen is appropriate or whether any doses

should be discontinued as well as whether the study should be stopped for futility. Enrollment will not be paused during the interim analysis.
If a dose level is dropped, participants who are in the DEP and have an iTD corresponding to a dropped dose will not be randomized into the RWP but will instead enter the OLE directly. In addition, participants who are in the OLE at a dose level that has been dropped will be transitioned to the highest dose level retained.
The size of the DEP interim analysis cohort and corresponding criteria for dropping a dose (see below) provide reasonable operating characteristics for proper determination if one or more doses should be dropped.

DMC Criteria for Dose Changes and Futility Evaluation by DMC	**Criteria to Drop the Dose**
1×10^{12} dose	< 20% of participants who attain an iTD are at the 1×10^{12} dose *OR* > 75% of participants who have 1×10^{12} as their iTD had a greater Phe reduction at their 6×10^{11} dose versus their 1×10^{12} dose
6×10^{11} dose Note: considered only if the Drop 1×10^{12} Criteria has been met	< 30% of participants who attain an iTD have 6×10^{11} or 1×10^{12} as their iTD *OR* > 75% of participants who have 6×10^{11} or 1×10^{12} as their iTD had a greater Phe reduction at their 3×10^{11} dose versus their 6×10^{11} dose
Stop for futility	< 30% of participants enrolled into the DEP attain an iTD and complete the DEP *OR* < 25% of participants who attain an iTD and complete the DEP are responders

DEP = dose-escalating, open-label period; DMC = data monitoring committee; iTD = individually titrated dose; Phe = phenylalanine

In addition, the DMC will review interim data at defined intervals in the study. The Sponsor will remain blinded to the subject-level blood Phe levels during the DEP and RWP but will have access to the DEP interim analysis results.

Multiplicity:

The overall Type I error rate for the DEP will be controlled at the [redacted]

1) Blood Phe level ≤ 360 µmol/mL at Week 4

2) Change from DEP baseline to Week 4 in blood Phe level

3) Percent change from DEP baseline to Week 4 in blood Phe level.

These key secondary endpoints will be tested following this prespecified order only if the RWP primary efficacy endpoint null hypothesis is rejected.

Safety Analysis:

Safety will be evaluated by scheduled monitoring of AEs, vital signs, and clinical laboratory measurements.

Adverse events will be coded using the *Medical Dictionary for Regulatory Activities*, and severity of AEs and laboratory abnormalities will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), v5.0, with certain exceptions from CTCAE grading for diarrhea and nausea. Adverse events will be tabulated by study period, system organ class, and preferred term. Incidence tables of participants with AEs will be presented for all AEs by maximum severity, AESIs, SAEs, AEs assessed as related to IMP, and AEs resulting in discontinuation of study dosing.

For the DEP, safety analyses will be performed on the DEP SAS. For the RWP and OLE study parts, safety analyses will be performed on the corresponding SAS, with results being presented for the responder and nonresponder populations as well as overall population.

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LIST OF ABBREVIATIONS

Abbreviation or Specialist Term	Explanation
AE	Adverse event
AraC	Arabinose-responsive transcriptional regulator
AUC	Area under the curve
BH_4	Tetrahydrobiopterin
BID	Twice per day
CBC	Complete blood count
CFU	Colony forming units
CRO	Contract research organization
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DAP	Diaminopimelate
DEP	Dose-escalating, open-label period
DMC	Data Monitoring Committee
ECG	Electrocardiogram
EcN	*Escherichia coli* Nissle 1917
eCRF	Electronic case report form
FAS	Full analysis set
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP	Good Laboratory Practice
IPTG	Isopropyl ß-D-1-thiogalactopyranoside
HA	Hippuric acid
HPA	Hyperphenylalaninemia
HV	Healthy volunteer
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IMP	Investigational medicinal product

Abbreviation or Specialist Term	Explanation
IRB	Institutional Review Board
iTD	Individually titrated dose
IXRS	Interactive response system
LAAD	L-amino acid deaminase
MAD	Multiple ascending dose
MMRM	Mixed-model with repeated measures
MTD	Maximum tolerated dose
NCI	National Cancer Institute
PAH	Phenylalanine hydroxylase
(m)PAL	(Modified) phenylalanine ammonia lyase
PD	Pharmacodynamics
Phe	Phenylalanine
PheP	High-affinity phenylalanine transporter
PK	Pharmacokinetics
PKU	Phenylketonuria
PP	Phenylpyruvate
PPI	Proton pump inhibitor
PPS	Per-protocol analysis set
QD	Once per day
qPCR	Quantitative polymerase chain reaction
REB	Research Ethics Board
RWP	Randomized withdrawal period
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SD	Standard deviation
SST	Stop signal task
$t_{½}$	Half-life
TCA	*Trans*-cinnamic acid
TEAE	Treatment-emergent adverse event
TID	Three times daily

Abbreviation or Specialist Term	Explanation
Tyr	Tyrosine
WOCBP	Women of childbearing potential

1. INTRODUCTION

The target indication for clinical development of SYNB1934v1 is for the treatment of phenylketonuria (PKU) in adult patients.

1.1. Background of Phenylketonuria

Phenylketonuria is a rare inherited metabolic disorder characterized by an inability to utilize the amino acid phenylalanine (Phe). The incidence of PKU varies widely among different populations with an incidence in the United States of 1:13,500 to 1:21,000.[1] There are pockets of high incidence, for example, in Ireland, where the incidence is 1:4,500 births. In the United States, newborn screening for PKU was initiated in the 1960s, which changed the natural history of the disease and resulted in improved outcomes for patients who were diagnosed in the neonatal period.[2]

PKU is caused by a mutation in the gene encoding phenylalanine hydroxylase (PAH), which is inherited in an autosomal recessive pattern and affects males and females equally. The PAH gene is located on chromosome 12q23.1. Tetrahydrobiopterin (BH_4) is a cofactor required for the activity of the PAH enzyme, and hyperphenylalaninemia (HPA) can also result from defects in the biosynthesis or recycling of BH_4. Phe, an essential amino acid, which is found in all protein-containing foods, is converted to tyrosine (Tyr) by PAH in the healthy population. PAH is either lacking or its activity is impaired in patients with PKU, leading to accumulation of Phe in the blood (a condition designated as HPA) and central nervous system and causing detrimental effects on brain development and function. Untreated, the disease results in severe neurological complications, including irreversible loss of cognitive capacity and parkinsonism.[3,4]

Phenylketonuria is diagnosed based on newborn screening. PKU may be classified by the degree of HPA, and patients presenting with plasma Phe levels > 1200 µmol/L have been described as having classical PKU.[6] Patients with mild to moderate PKU present with Phe serum concentrations ranging from 360 to 1200 µmol/L, and benign HPA is characterized by the ability to maintain plasma Phe-below the range required for treatment.[6] A widely accepted treatment goal is to achieve a blood Phe level of 120 to 360 µmol/L.[7] A majority of specialized centers initiate treatment at a baseline plasma Phe level of > 360 µmol/L, although other centers initiate treatment at 600 µmol/L.

Blood Phe lowering is the goal of therapy. If PKU is detected early and treatment is started within first weeks of life, intellectual disability can be prevented.[8] Current recommendations require lifelong dietary support, with intensification during preconception and pregnancy for women.[7] According to US-based treatment guidelines, the goal of PKU treatment is to maintain the blood concentrations of Phe between 120 and 360 µmol/L for all patients regardless of age.[7]

Allelic variation in the PAH locus leads to a wide range of phenotypes and variation in Phe level.[10] Treatment and maintenance of metabolic control throughout life is essential to optimal functioning of PKU patients. While intellectual disability does not occur in patients who are well controlled in infancy and childhood, a variety of adverse neurocognitive and psychiatric outcomes, including deficits in executive functioning and psychiatric symptoms such as anxiety, depression, and phobias can develop later in life when Phe control is relaxed. High Phe is associated with an increased prevalence of neuropsychiatric symptoms and executive functioning

deficits, whereas low Phe is associated with improved neurological performance.[11,12] Even in well-controlled patients with normal IQ, some impairment of intellectual abilities has been observed.[13] During childhood, each 100 µmol/L increase in blood Phe predicted a 1.3- to 3.1-point reduction in IQ over a range of Phe levels of 423 to 750 µmol/L. When imputed over lifetime Phe levels, the 100 µmol/L increase in Phe predicted a 1.9 to 4.1 reduction in IQ.[14] Even small, short term (~200 µmol/L, 1–2 weeks) shifts in blood Phe levels can impact outcomes of working memory and sustained attention.[15]

1.2. Unmet Medical Need in Phenylketonuria

Current standard treatment for patients with PKU is a stringent Phe-restricted diet, combined with amino acid mixtures supplemented with trace elements to prevent nutritional deficiencies.[16,17] This is achieved by excluding or severely curtailing protein-containing foods and providing a protein supplement that has other amino acids, but not Phe (medical food, "formula"), along with frequent monitoring of blood Phe levels.[16] Most patients with classic PKU tolerate < 500 mg Phe per day (10 g natural protein), while patients with mild to moderate PKU tolerate < 1000 mg Phe per day (20 g natural protein).[16] While in the past it was thought that strict dietary control was only required in early childhood, current recommendations require lifelong dietary support with intensification during preconception and pregnancy for women, given the risk of teratogenic effects of elevated blood Phe on the developing fetus.[18]

Two therapeutics for PKU have been approved by the Food and Drug Administration (FDA), sapropterin dihydrochloride (Kuvan®) and pegvaliase (Palynziq®), which are described below.

Kuvan is a synthetic form of the BH_4 cofactor of the PAH. The BH_4 cofactor increases the activity level of the PAH enzyme and increases the amount of Phe that can be converted to Tyr. Kuvan is indicated for the treatment of HPA in adults and pediatric patients of all ages with PKU who have been shown to be responsive to such treatment and also for the treatment of HPA in adults and pediatric patients of all ages with BH_4 deficiency who have been shown to be responsive to such treatment (Kuvan, Union Register EU/1/08/481, last updated 31 March 2021).[19] Approximately 25% to 50% of PKU patients are sapropterin-responsive.[20]

Palynziq is a Phe-metabolizing enzyme that is composed of recombinant phenylalanine ammonia lyase (PAL) (rAvPAL) conjugated to *N*-hydroxysuccinimide -methoxypolyethylene glycol. It is indicated for the treatment of patients with PKU aged 16 years and older who have inadequate blood Phe control (blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options (Palynziq, Union Register, EU/1/19/1362, last updated 31 May 2021).[21] Palynziq is dosed as daily subcutaneous injections. Anaphylaxis has been reported after administration of Palynziq and may occur at any time during treatment.

There remains a significant unmet medical need despite existing therapies. Strict blood Phe control by dietary management is challenging and poses a significant burden on the patients.[22] Despite recommendations supporting life-long control of Phe levels, some children and most adults cannot comply due to the highly restrictive nature of the diet.[23] This puts these patients at risk of cognitive and psychiatric disease and supports the need for novel treatment approaches. Adult patients who are no longer on a Phe-restricted diet can experience neurological complications that can improve or even reverse after reinstitution of treatment.[7] Based on clinical

experience and research data, adults and older adolescents are less adherent to a strict Phe-restricted diet than younger children, who are under their parents' control.[8]

1.3. Description of SYNB1934 and SYNB1934v1

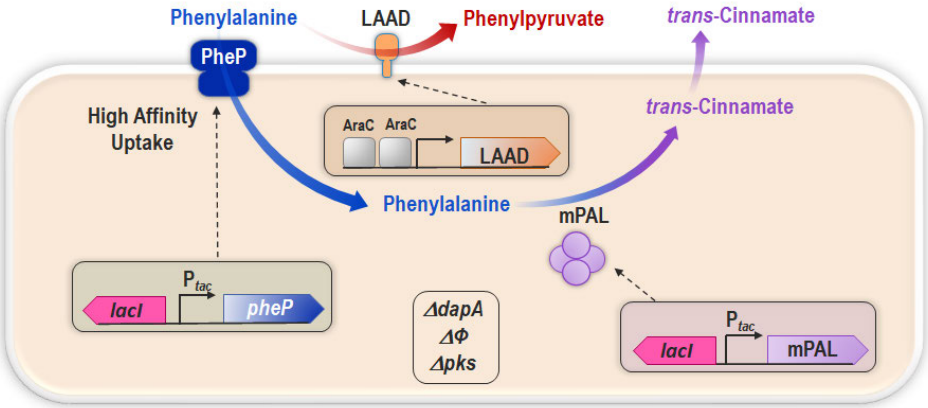
SYNB1934 was derived from *Escherichia coli* Nissle 1917 (EcN) in a series of genetic manipulations designed to allow enhanced degradation of Phe within the human gut. The degradation of Phe is carried out by the activity of engineered genes encoding phenylalanine ammonia lyase (PAL), which catalyzes the conversion of Phe to *trans*-cinnamic acid (TCA), and L-amino acid deaminase (LAAD), which catalyzes the conversion of Phe to phenylpyruvate (PP). The PAL expressed in SYNB1934 was derived from *Photorhabdus luminescens* (recently reclassified as *Photorhabdus laumondii subsp. laumondii*), the same PAL utilized in the closely related strain SYNB1618. However, in SYNB1934, this PAL differs from the wild-type PAL sequence at 5 amino acid positions. This modified PAL (mPAL) was identified in a high throughput screening campaign that selected for PAL enzyme variants with higher rates of TCA production compared to the wild-type PAL. Additionally, SYNB1934 contains the following modifications within the genome of EcN that are intended to enhance Phe degradation while augmenting biologic containment through diaminopimelate (DAP) auxotrophy:

a. Insertion of 1 additional copy of an endogenous Nissle gene encoding a high-affinity phenylalanine transporter (PheP) under the regulatory control of an isopropyl ß-D-1-thiogalactopyranoside (IPTG)-inducible promoter (P_{tac}) and transcriptional repressor LacI
b. Insertion of 4 copies of a gene encoding mPAL under the regulatory control of P_{tac} and LacI
c. Insertion of the gene encoding L-amino acid deaminase (LAAD) derived from *Proteus mirabilis* under the regulatory control of the arabinose-inducible promoter P_{BAD} and the arabinose-responsive transcriptional activator (AraC)
d. Deletion of the *dapA* gene that encodes 4-hydroxy-tetrahydropicolinate synthase to create an auxotrophy for the essential cell wall component DAP
e. Inactivation of the endogenous Nissle prophage
f. Deletion of the polyketide synthase (*pks*) island containing the colibactin-production genes

The deletion of the *pks* island resulted in a modified strain of SYNB1934, which is referred to as SYNB1934v1.

A schematic of SYNB1934v1 is shown in Figure 1.

Figure 1: Schematic of SYNB1934v1



AraC = arabinose-responsive transcriptional regulator; LAAD = L-amino acid deaminase from *Proteus mirabilis*; mPAL = gene encoding phenylalanine ammonia lyase from *Photorhabdus luminescens* with the following engineered mutations: S92G, H133M, I167K, L432I, V470A; *pheP* = high affinity phenylalanine transporter; P_{tac} = synthetic promoter controlled by LacI; Δ*dapA* = deletion of *dapA* gene leading to diaminopimelate auxotrophy; *ΔΦ* = partial deletion of endogenous Nissle prophage; *Δpks* = deletion of the genetic island responsible for the production of colibactin

1.4. Nonclinical Studies

The pharmacologic activity of SYNB1934v1 and its closely related strain, SYNB1618, has been characterized in a series of non-Good Laboratory Practices (GLP) studies in mice and nonhuman primates that demonstrate the in vitro and in vivo Phe-consuming activity of the strain, as well as dose response, biodistribution, excretion, and pH sensitivity. The safety of SYNB1618 has been evaluated in a 28-day GLP toxicology study in mice. Further details on the nonclinical studies can be found in the Investigator's Brochure.

1.5. Clinical Experience

SYNB1934 has been investigated in 2 clinical studies, SYNB1934-CP-001 and SYNB1618-CP-003. The related strain SYNB1618 has been investigated in 3 completed studies, SYNB1618-CP-001, SYNB1618-CP-002, and SYNB1618-CP-003.

1.5.1. Study SYNB1934-CP-001

SYNB1934-CP-001, a Phase 1, dose-escalation, placebo- and active-controlled crossover study, assessed the safety, tolerability, and pharmacodynamics of SYNB1934 (an engineered EcN closely related to SYNB1618) and [redacted] in healthy volunteers. Part 1 was a multiple-ascending dose design in which participants were dosed with either SYNB1934, SYNB1618, or placebo. In Cohort 2 of Part 1, participants received SYNB1934 or SYNB1618 or placebo in the first treatment period. After a ≥ 7-day washout period after the first treatment period, they crossed over to the alternate EcN strain in the second treatment period (or remained on placebo). [redacted] Forty subjects were dosed in Part 1.

Part 2 was an open-label, randomized sequence, crossover study of SYNB1934 [redacted] Twenty-two subjects were dosed in Part 2.

The maximum tolerated dose was not reached. The highest dose administered was 2×10^{12} SYNB1934. Dose-related decreases in labeled Phe area under the curve (AUC), as well as increases in the Phe metabolites, TCA and HA, were observed.

No deaths, serious adverse reactions, or SAEs occurred. The most commonly reported AEs were nonserious gastrointestinal (GI)-related AEs, which are a known potential risk of EcN. A dose-related increase in the number and severity of GI-related AEs was observed. The majority of AEs were mild or moderate. Four participants discontinued IMP due to an AE. No clinically significant changes in laboratory values or vital signs have been observed.

Data on clearance of SYNB1934 from this study have confirmed the expected time to clearance from the GI tract, with no participant with a positive fecal quantitative polymerase chain reaction result more than 1 week after the last dose. There were no signs of colonization, and no participants have required antibiotics for clearance.

1.5.2. Studies of SYNB1618

Study SYNB1618-CP-001 was a Phase 1/2a, first-in-human, oral, single and multiple dose-escalation, randomized, double-blinded, placebo-controlled study of SYNB1618 in healthy volunteers (HVs) and adult participants with PKU to evaluate the safety, tolerability, kinetics and pharmacodynamics (PD) of the frozen liquid formulation. The study consisted of 2 parts: a single-ascending dose (SAD) study and a multiple-ascending dose (MAD) study.

The study enrolled 56 HVs and 14 PKU participants, all of whom received at least one dose of SYNB1618 or placebo. Dose-related increase in strain-specific biomarkers of Phe metabolism (D5-labeled and unlabeled plasma TCA and urine hippuric acid [HA]) were observed in both HV and PKU active treatment groups compared to placebo in both SAD and MAD study parts.

No treatment-related serious adverse events (SAEs) or systemic toxicity or infections were observed. The majority of treatment-emergent adverse events (TEAEs) were either mild or moderate in severity and reversible. Most TEAEs were GI-related and similar to those described for the parent strain EcN (i.e., abdominal pain, gut noises, loose stools/diarrhea, nausea, vomiting, and headache). At increasing doses, GI related TEAEs, mainly nausea and vomiting, were dose limiting. One HV in the highest MAD cohort (1×10^{11} colony forming units [CFU]) discontinued dosing due to Grade 2 nausea that was considered related to treatment.

All participants cleared the bacteria following discontinuation of dosing. The single-dose MTD was defined as 2×10^{11} CFU.

SYNB1618-CP-002 was conducted in adult male and female HVs to bridge the safety, tolerability, and PD of SYNB1618, using the lyophilized powder for oral suspension. This study included a tracer study and a biomarker study to assess levels of Phe and its metabolites. In total, 88 subjects were enrolled.

Dose-dependent reductions in D5-Phe AUC were observed in subjects administered SYNB1618 compared with placebo subjects after the protein load in the tracer study. In addition, dose-related increase in strain-specific biomarkers of Phe metabolism (D5-labeled and unlabeled

plasma TCA and urine HA) were observed. In the biomarker study, the production of TCA in the fasted state was similar in magnitude to the production after a protein load in the SYNB1618-treated subjects. No TCA production was observed in the placebo group. This supports strain activity in the fasted state, regardless of dietary Phe intake.

No treatment-related SAEs were reported. As in the SYNB1618-CP-001 study, mild to moderate GI AEs were observed in a dose-related fashion. Nausea and vomiting were the dose-limiting symptoms. The MTD was determined to be 2×10^{12} live cells TID. A small, dose-related increase in C-reactive protein (CRP) was observed in some participants; the clinical significance of this finding is unclear, and no AEs appeared to be related to CRP increases. The CRP level returned to normal after the end of dosing in those participants who had a follow-up sample available.

SYNB1618-CP-003 (SynPheny-1) was a Phase 2, open-label study of the efficacy and safety of SYNB1618 and SYNB1934 in participants with PKU, designed as a proof-of-concept study to show Phe lowering, relative to baseline. This 2-arm study used a dose-ramp regimen consisting of 4 dose levels of SYNB1618 (1×10^{11}, 3×10^{11}, 1×10^{12}, and 2×10^{12} live cells) over 15 days of treatment in Arm 1, and 4 dose levels of SYNB1934 (3×10^{11}, 6×10^{11}, 1×10^{12}, and 2×10^{12} live cells) over 15 days of treatment in Arm 2. The treatment period was preceded by a 6-day diet run-in period, with concomitant PPI administration throughout the treatment period. A tracer study, using D5-labeled Phe, and a biomarker study were conducted.

Interim data from Arm 1 of the study and preliminary data from Arm 2 of the study support the Phe-lowering capacity of SYNB1618 and SYNB1934, respectively. Nine out of 15 PKU participants (60%) had at least a 20% reduction in fasting Phe. Of these 9 participants, 7 participants demonstrated Phe lowering below the guidance threshold for adults with PKU of 600 μmol/L, and 2 participants had levels ≤ 360 μmol/L, including a single participant reaching a normal physiological Phe level. Evaluation of labeled and unlabeled plasma TCA and urinary HA supported findings of the primary and secondary efficacy analysis: Levels of these metabolites increased in a dose-related manner during the treatment period, demonstrating breakdown of Phe in the GI tract.

No deaths, serious adverse reactions, or treatment-related SAEs have been reported. Two participants in Arm 1 experienced increased levels of CRP on Day 14; these levels had returned to normal at follow-up on Day 29. One participant in Arm 2 experienced increased CRP on an unscheduled visit; these levels had returned to normal at follow-up on Day 29. The clinical significance of this finding, if any, is unclear. No other potentially clinically significant laboratory values or clinically significant changes in vital signs measurements have been reported.

1.6. Study Rationale

Due to the restrictive nature of the diet and limitations of current treatment, there remains an unmet medical need for new treatments for people with PKU. Given the favorable safety profile of SYNB1934 and the closely related strain SYNB1618 to date, gut-restricted mechanism of action, and demonstrated potential for clinical benefit as shown by blood Phe lowering in PKU participants in study SYNB1618-CP-003, further clinical development of SYNB1934v1 as a potential treatment for PKU is warranted.

PKU participants on sapropterin who do not reach the treatment target of blood Phe ≤ 360 µmol/L could benefit from addition of SYNB1934v1 in the management of blood Phe. Given the differing mechanisms of action of SYNB1934v1 and sapropterin, and different sites of action (sapropterin acting intracellularly in the liver on PAH activity, and SYNB1934v1 acting locally in the GI tract on Phe degradation), interaction between these 2 agents should not be anticipated. Participants on sapropterin enrolled in prior studies have had similar safety and PD compared to the overall population. Participants on a stable dose of sapropterin and stable diet, with blood Phe > 360 µmol/L are eligible for this study; therefore, any additional blood Phe lowering in SYNB1934v1-treated participants could be attributed to the IMP.

Based on the mechanism of action of the strain and data in prior clinical studies, there is a linear relationship between the dose and Phe metabolism, which has not reached a plateau at the maximum dose studied (2×10^{12} live cells). Dosing is limited by GI tolerability which appears to be variable across individuals. Titration of dose over time has led to improved tolerability. Blood Phe lowering has been demonstrated at a dose of 3×10^{11} live cells with the closely related strain SYNB1618, and this dose will be the lowest potential iTD for the study. Data from study SYNB1618-CP-003 show that a higher dose leads to increased Phe lowering potential and thus, improved clinical benefit. Therefore, in dose-escalating, open-label period (DEP) participants will titrate to their individual titrated dose (iTD) over time guided by AEs in order to achieve the maximum clinical benefit

Part 2, the randomized withdrawal period (RWP), will allow blinded comparison to placebo while participants maintain a stable diet. Phe half-life ($t_{1/2}$) in PKU is 24 hours, and a steady state is reached within 5 days. The 4-week duration in RWP will minimize time off active treatment while allowing blinded comparison to placebo.

Response to SYNB1934v1 varies based on individual factors related to GI physiology (e.g., gastric pH, GI transit time) as well as total intake of dietary Phe. Subjects achieving ≥ 20% blood Phe lowering in DEP will be considered responders and will be included in the primary efficacy analysis population. A 20% change in biomarker level exceeds assay variability and can generally be considered biologically meaningful and was therefore selected as the threshold for responder definition.

The OLE will enable collection of long-term safety and efficacy data for up to 36 months. Dietary intake of Phe may be modified during the OLE based on measured blood Phe lowering if the recommended level of Phe < 240 µmol/L has been achieved. Dietary liberalization of natural protein while maintaining controlled Phe levels is of benefit to the participants and facilitates dietary compliance.

1.6.1. Enrichment Design

The trial will employ an enrichment strategy of having PKU participants who have a ≥ 20% reduction from baseline in blood Phe during DEP (“responders”) be the primary analysis population in the RWP portion of the study. This strategy is consistent with the predictive enrichment strategy discussed in the FDA guidance on enrichment strategies.[24] As discussed in the guidance, this enrichment strategy chooses participants who are more likely to respond to the drug treatment than other participants with the condition being treated. The objective of predictive enrichment is to increase study efficiency/feasibility by having a larger effect size and

use of a smaller study population and provide an enhanced benefit-risk relationship for the responder participants compared to the overall population.

The criteria for the enriched population, a ≥ 20% reduction from baseline in blood Phe, is justified in Section 1.6. This enrichment approach is consistent with similar approved therapies for PKU (Kuvan and Palynziq).

As the final blood Phe assessments from the DEP will not be available at the time of randomization, all participants who complete the DEP will be randomized into the RWP. Although responder participants will be the primary analysis population, the nonresponders will also be included in the RWP portion of the study as well as the OLE of the study. This will allow for the assessment of the treatment effect and safety profile in the nonresponder population.

1.7. Benefit-Risk Assessment

SYNB1934 and its closely related strain SYNB1618 have been studied in more than 230 HVs and PKU participants. The safety profile has been consistent with the probiotic chassis EcN, which has been used in participants for decades in the European Union and Canada without requirement for prescription. There have been no treatment-related SAEs, no deaths, no systemic toxicity, and no infections. Interindividual variability in tolerability has been observed. The majority of TEAEs have been either mild or moderate in severity and reversible. Most AEs have been GI-related and included GI discomfort, nausea, and vomiting. At increasing doses, GI-related AEs, mainly nausea and vomiting, have been dose limiting. A small dose-related and reversible increase in CRP has been observed in some participants; the clinical significance of this finding is unclear. The CRP level has returned to normal after end of dosing.

Consistent with the design of SYNB1934, clearance is rapid following discontinuation of dosing. In study SYNB1618-CP-001, SYNB1618 was cleared within 4 days following the last dose, as indicated by fecal quantitative polymerase chain reaction (qPCR) analyses. Data from study SYNB1618-CP-002 are consistent. Across all study parts, following up to 7 days of repeat dosing with SYNB1618, no fecal sample was above the limit of quantification at 7 days after last dose. In neither study was there any evidence of colonization, nor did any participant require antibiotic treatment to achieve clearance. Clearance data for SYNB1934 are consistent with that for SYNB1618 with no subject having a positive qPCR sample more than 1 week after the last dose.

Data from completed studies demonstrates that SYNB1934 can access Phe from within the GI tract, as demonstrated by the D5-Phe meal challenge. A dose-related decrease in plasma D5-Phe AUC has been observed, with corresponding increase in strain-specific biomarkers plasma D5-TCA and urinary D5-HA. In the Phase 2 study SYNB1618-CP-003, SYNB1934 led to a mean 34% decrease in plasma Phe in PKU participants, thus demonstrating potential for clinical benefit in the treatment of PKU. Individual variability in Phe lowering was observed. Data from HVs in SYNB1934-CP-001 demonstrate increased Phe conversion capacity of SYNB1934 compared to SYNB1618 based on strain-specific biomarkers.

The risk of unintended effects of using genetically engineered EcN is considered low. The products of the 2 engineered Phe degradation pathways are TCA, produced via the PAL pathway, and PP, produced by the deamination of Phe by LAAD. TCA is a naturally occurring compound and is found in several plants that are consumed as food. It is frequently added to food

as a flavor ingredient and is generally regarded as safe.[25] Phenylpyruvate is normally present in low amounts in human blood and urine but in patients with PKU who lack PAH enzyme activity, large quantities can be excreted in the urine. It is not associated with any known toxicity. An additional on-target toxicity risk is related to lowering of blood Phe levels below the normal range. However, low blood Phe levels could be tolerated without significant sequelae for at least 2 months based on clinical observations in prior PKU trials.[23] Blood Phe levels will be monitored throughout the study and measures are implemented to correct Phe levels if they fall below the acceptable range.

Overall, the benefit–risk assessment for SYNB1934 supports initiation of this Phase 3 trial to evaluate the safety and efficacy of SYNB1934v1 in a larger cohort of PKU participants.

2. STUDY OBJECTIVES AND ENDPOINTS

This study has 3 parts, and each part has a distinct set of objectives, endpoints, and analysis populations. The determination of the primary evidence of efficacy will be the comparison of SYNB1934v1 versus placebo in the randomized withdrawal period (Part 2).

Objectives and endpoints are presented in Table 1 to Table 3 for the respective parts of the study.

Table 1: Objectives and Endpoints: Part 1, Dose Escalating, Open-Label Period (DEP)

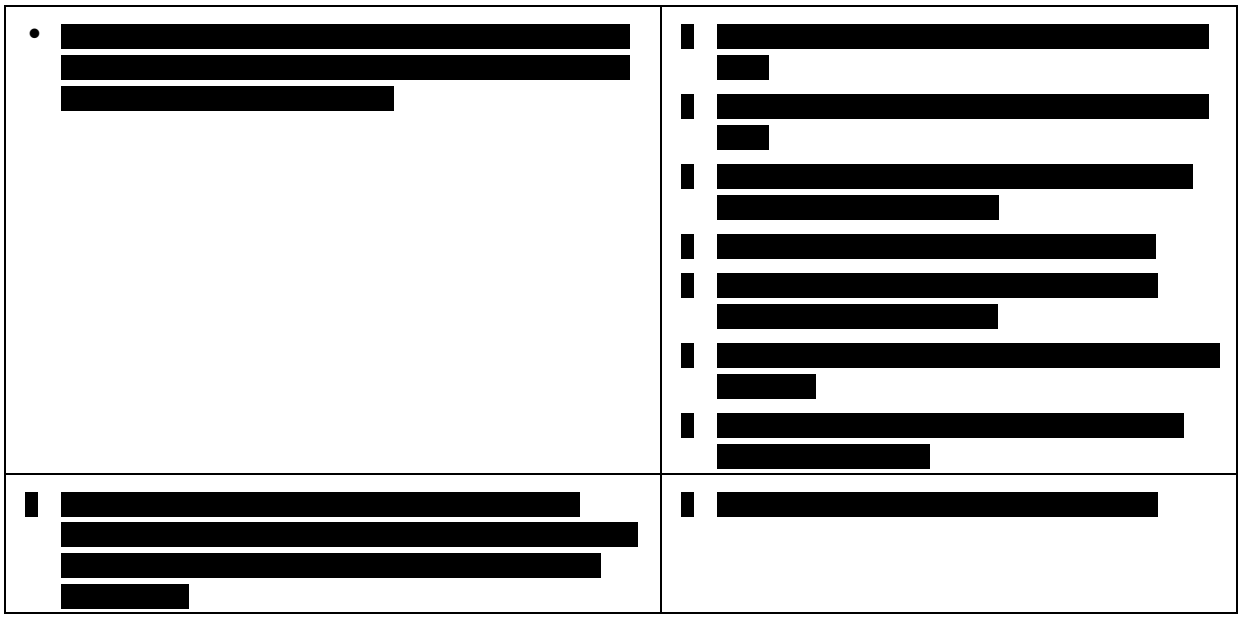
Objective	Endpoint
Primary: To assess the percent change in blood Phe level	• Percent change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1
Key Secondary:	
• To assess the absolute change in Phe level	• Change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1
• To determine the responder population	• A ≥ 20% reduction from baseline in blood Phe level at any time in the DEP
Other Secondary:	
• To assess proportion of participants achieving blood Phe level ≤ 360 µmol/L	• Blood Phe level ≤ 360 µmol/L at any time of the iTD of SYNB1934v1 • Blood Phe level ≤ 360 µmol/L at any time in the DEP
• To assess the safety and tolerability of SYNB1934v1	• Incidence and severity of adverse events • Changes from baseline in clinical laboratory parameters • Changes from baseline in vital signs measurements
Exploratory:	
• [redacted]	[redacted]

Objective	Endpoint
• [redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Note: baseline and week numbering are specific to study Part 1, unless explicitly noted otherwise in the endpoint.

Table 2: Objectives and Endpoints: Part 2, Randomized Withdrawal Period (RWP)

Objective	Endpoint
Primary: To evaluate efficacy of SYNB1934v1 versus placebo in the responder population	• Change from baseline to Week 4 in blood Phe level
Key Secondary:	
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to percent change from DEP baseline in blood Phe level	• Percent change from DEP baseline in blood Phe level at Week 4
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to change from DEP baseline in blood Phe level	• Change from DEP baseline to Week 4 in blood Phe level
• To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to the proportion of participants with a blood Phe level ≤ 360 µmol/L	• Blood Phe level ≤ 360 µmol/L at Week 4
Other Secondary Objectives:	
• To assess the safety and tolerability of SYNB1934v1	• Incidence and severity of adverse events • Changes from baseline in clinical laboratory parameters • Change from baseline in vital signs measurements
• To evaluate efficacy of SYNB1934v1 versus placebo in the responder population with regard to the proportion of participants with a ≥ 20% reduction from DEP baseline in blood Phe level at Week 4	• A ≥ 20% reduction from DEP baseline in blood Phe level at Week 4
Exploratory:	
• [redacted]	[redacted]



Note: baseline and week numbering are specific to study Part 2, unless explicitly noted otherwise in the endpoint.

Table 3: Objectives and Endpoints: Part 3, Open-label Extension (OLE)

Objective	Endpoint
• To assess the safety and tolerability of SYNB1934v1	• Incidence and severity of adverse events • Changes from DEP baseline in clinical laboratory parameters • Changes from DEP baseline in vital signs measurements
• To assess absolute and relative change in blood Phe level in the responder and nonresponder populations	• Change from DEP baseline in blood Phe levels • Blood Phe level ≤ 360 µmol/L
• To assess impact of SYNB1934v1 and dietary protein intake on Phe levels	• Change from DEP baseline in blood Phe levels over time • Change from DEP baseline in blood Phe level over time until first protocol-specified increase in dietary protein intake
• To assess impact of SYNB1934v1 on dietary Phe intake	• Change from DEP baseline in dietary Phe intake

Note: baseline and week numbering are specific to study Part 3, unless explicitly noted otherwise in the endpoint.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This is a 3-part, adaptive study consisting of a dose-escalating, open-label period (DEP; Part 1) of up to 15 weeks, followed by a 4-week, double-blind, placebo-controlled, randomized withdrawal period (RWP; Part 2), and an open-label extension (OLE; Part 3) of up to 36 months. Figure 2 presents a study schematic.

In the DEP, all enrolled participants will maintain a stable diet reflecting their baseline Phe intake and receive escalating doses of SYNB1934v1 from approximately 3 to 15 weeks to determine an iTD, which is defined as the highest dose the participant is able to tolerate. A participant will be defined as having reached an iTD if they tolerate 3 weeks at a dose, regardless of whether other doses are tolerated.

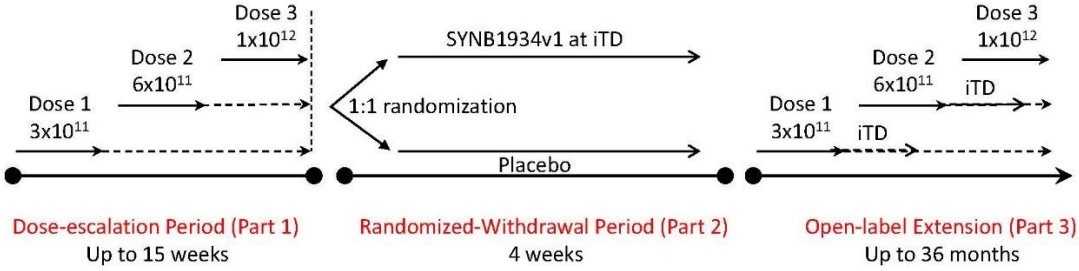
Blood Phe level will be measured at each dose level after 3 weeks at that level. A responder will be defined as a participant who achieves a ≥ 20% reduction in blood Phe level compared to DEP baseline on SYNB1934v1.

The DEP will contain an optional substudy (DEP weekly substudy) [redacted] Additional values obtained as part of the substudy will not be used to determine responder status.

Participants who complete at least 3 weeks at their iTD during the DEP will enter a 4-week RWP. Participants will be randomized 1:1 to receive SYNB1934v1 at their iTD determined in the DEP or placebo TID. Randomization will be stratified on screening Phe level. Blood Phe level will be measured at Weeks 1, 3, and 4 of the RWP. Participants will remain on the same diet they consumed during the DEP.

Participants who complete the 4-week RWP may enter the OLE and receive SYNB1934v1 for up to 36 months at their iTD. (At the sponsor's discretion, enrollment of new participants directly into OLE may be opened.) The iTD in Parts 1 and 3 can differ. Participants will be allowed to modify their standard diet if their blood Phe level is < 240 µmol/L, as determined by either local or central laboratory testing, with guidance from the investigator as outlined in the study-specific Diet Manual. The 3-day dietary intake assessments will be conducted per the Schedule of Events (Table 5).

Figure 2: Study SYNB1934-CP-003 Schematic



iTD = individually titrated dose. Should the DMC remove doses from the study after completion of the interim analysis, the dose ramp will be altered to reflect these changes.

3.2. Dosing and Dose Escalation

SYNB1934v1 will be taken with a meal throughout the study. Participants will take a proton pump inhibitor (PPI) once per day (QD), recommended to occur before breakfast, during all parts of the study (see Section 4.3.4), starting at Day –7 of the DEP. If participants are unable to tolerate the PPI, they may attempt to use an H2 blocker per investigator discretion. If neither the PPI nor H2 blocker can be tolerated, the participant will discontinue from the study.

3.2.1. Part 1: Dose-Escalating Open-Label

In the DEP, participants will complete a dose ramp at the beginning of each dose level. During the dose ramp, SYNB1934v1 will be administered as shown in Table 4. Dosing will be with meals. On days with QD dosing, SYNB1934v1 should be taken with the mid-day meal. On days with twice per day (BID) dosing, SYNB1934v1 should be taken with the morning and evening meal.

Table 4: Dose Ramp Schedule

	Dose Level 1			**Dose Level 2**			**Dose Level 3**		
Timing	D1-9	D10-14	D15-21	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9
Dose (live cells)	[redacted] 3×10^{11}*	3×10^{11}	3×10^{11}	6×10^{11}	6×10^{11}	6×10^{11}	1×10^{12}	1×10^{12}	1×10^{12}
Frequency	QD, D1-3	QD, D10-11	TID	QD × 3 days	TID	TID	QD × 3 days	TID	TID
	BID, D4-6	BID, D12-14		BID × 4 days			BID × 4 days		
	TID, D7-9								

BID = twice per day; D = day; QD = once per day; TID = 3 times per day.
*See Section 4.3.1

[redacted]

Participants will dose escalate to their iTD based on tolerability. If the participant tolerates the 3×10^{11} dose after 3 weeks, and the investigator believes that further tolerance is likely, the investigator will escalate to the 6×10^{11} dose. If the 6×10^{11} is tolerated, the participant will continue with this dose for a total duration of 3 weeks. If the participant tolerates the 6×10^{11} after 3 weeks, and the investigator believes that further tolerance is likely, the investigator will escalate to the 1×10^{12} dose. The investigator can, with the medical monitor's assent, declare that the participant has reached iTD at any point during dose escalation, but the participant must stay on his or her iTD for 3 weeks prior to entering RWP.

A subset of the population will participate in the DEP weekly substudy, intended to enroll 20 [redacted]
Participants in this substudy will have [redacted]. Additional values obtained as part of the substudy will not be used to determine responder status.

3.2.1.1. Intolerance

Dose-related GI TEAEs have been seen with SYNB1934 in prior studies. These events have been most likely to occur with an escalation of dose and can often be addressed by treatment with concomitant medications (e.g., ondansetron) and/or dose reduction. If at any time during a 3-week dosing interval the participant or investigator considers a dose level to be intolerable, the following actions may be taken:

- Start [redacted] the investigator's discretion [redacted] as needed).

- Decrease the frequency of dosing for 1 to 2 days. This can be initiated without the approval of the medical monitor.
- Skip dosing 1 to 2 days. This can be initiated without the approval of the medical monitor.
- Skip dosing ≥ 3 days. This must be discussed with the medical monitor.
- De-escalate to a previous level after discussion with the medical monitor. In this case, the participant will restart 3 weeks at a previously tolerated dose including its ramp, and this previously tolerated dose will be the iTD. After de-escalation, participants cannot escalate to a higher dose again during Part 1.

In general, a dose is considered intolerable if the participant experiences Grade 3 or more severe TEAEs or Grade 2 GI AEs that do not improve with continued dosing at the same dose level over time. If a dose is de-escalated due to a reason other than Grade 3 or greater or Grade 2 GI TEAEs, or a second de-escalation is required, the medical monitor must be consulted. Participants must take at least 8 of the 9 expected doses on the final 3 days of their iTD period. If not, the medical monitor must be consulted before the participant enters Part 2.

3.2.2. Part 2: Randomized Withdrawal

In the RWP, participants who completed DEP will be randomized 1:1 to receive either SYNB1934v1 at their iTD established in the DEP or placebo. Participants will remain on this dose of investigational medicinal product (IMP; i.e., SYNB1934v1 or placebo) for the duration of the RWP, unless they develop intolerance or meet other discontinuation criteria (see Section 3.5). Doses of SYNB1934v1 cannot be modified during the RWP.

3.2.3. Part 3: Open-Label Extension

During the OLE, all participants will complete a dose ramp to their iTD over time guided by tolerability, as described for the DEP, including the full dose ramp schedule (Table 4). The iTD in OLE may be different from the iTD in DEP or RWP. The investigator may escalate the dose of SYNB1934v1 up to a maximum of 1×10^{12} live cells based on tolerability; multiple attempts to escalate to a higher dose level are permitted per investigator discretion.

3.3. Diet and Phenylalanine Monitoring

For 3 consecutive days prior to scheduled study visits and blood Phe draws, all participants will have a 3-day dietary intake assessment (see Table 5). A study dietitian will interview the participant and provide advice on maintaining a diet that is consistent with baseline diet throughout the study. Participants will follow their usual diet (including Phe, protein, and medical food intake) from baseline in the DEP to the end of the RWP.

During the DEP and RWP, blood Phe levels will be blinded to the sponsor, participant and investigator. The investigator will be notified by the central laboratory if Phe is > 1.5 × the DEP baseline value, if Phe falls below 30 µmol/L or if Tyr falls below 20 µmol/L, based on a defined monitoring plan (to be provided in the Study Manual; see also Section 8.11). During the OLE, participants will be allowed to modify their standard diet if their blood Phe level is < 240 µmol/L, as determined by either local or central lab, with guidance from the investigator as

outlined in the study-specific Diet Manual. The 3-day dietary intake assessments will continue per the Schedule of Events (Table 6).

3.4. Study Stopping Rules

If any of the following criteria are met, enrollment will be halted. Participants already enrolled in the study will continue dosing until review by the Data Monitoring Committee (DMC).

1. Two or more participants experience an AE ≥ Grade 3, per protocol severity grading criteria, related to SYNB1934v1 as assessed by the investigator.
2. Clinical infection is detected in a sterile space with a gram-negative aerobic bacillus confirmed by qPCR to be SYNB1934v1.

If any of the above criteria are met, the DMC will review all relevant data and determine whether enrollment in the study should resume and whether any modifications to the study are warranted. If the DMC decides that further enrollment should be permanently suspended, subjects already enrolled in the study will continue to be followed for the duration of the study.

If a death occurs at any time during the study that is considered by the investigator to be related to SYNB1934v1, enrollment will be halted and dosing for subjects already enrolled will be stopped until the DMC reviews all relevant data and makes further recommendations.

This study may also be discontinued at any time due to safety concerns (including, but not limited to, the stopping rules described below), failure to meet expected enrollment goals, administrative reasons, or at the discretion of the sponsor. Should the study be terminated prematurely, the sponsor will provide written notification to the investigator and regulatory authorities and will specify the reasons for early termination. The investigator must inform the Institutional Review Board (IRB), Independent Ethics Committee (IEC), or Research Ethics Board (REB) promptly and provide the reasons for the termination.

3.5. Study Discontinuation for Individual Participants

Reasons for discontinuation of IMP may include the following:

- The participant withdraws consent.
- The investigator or sponsor notes a significant noncompliance with protocol procedures (e.g., use of a prohibited concomitant medication).
- The participant develops an intolerable toxicity including but not limited to a Grade 3 or more severe AE or SAE assessed as related to IMP by the investigator.
- The investigator determines that the participant must discontinue further study dosing for medical reasons (including pregnancy).
- The participant has low levels of Phe (< 30 μmol/L) or Tyr (< 20 μmol/L) or has profound elevations in Phe (>1.5 × the DEP baseline value during DEP and RWP and > 2200μM during OLE).

If possible, the investigator will confer with the sponsor or medical monitor before discontinuing dosing. Participants who discontinue from IMP in RWP will be encouraged to remain on study

and complete assessments (particularly safety assessments) through the remainder of RWP. Participants who discontinue from DEP and OLE will be asked to have an early termination visit 30 days after the last dose of IMP (see Section 5.6).

Participants may withdraw their consent at any time for any reason without prejudice to their future medical care by the physician or at the institution. If a participant withdraws consent, the date and stated reason for consent withdrawal should be documented. Participant data collected up to the date of consent withdrawal will be included in the analyses.

3.6. Duration of Study Participation

The maximum time of study participation for a participant is planned to be approximately 6 months for screening through RWP and up to 36 months for Part 3:

- Screening: up to 45 days
- Part 1 (DEP): up to 15 weeks
- Part 2 (RWP): 4 weeks
- Part 3 (OLE): up to 36 months

The clinical trial concludes with the final visit of the OLE (36 months). No additional arrangements are made for the care of subjects following the completion of the trial, as they will be returned to the standard of care for PKU.

4. STUDY POPULATION

4.1. Number of Participants

This study plans to enroll [redacted]. More participants may be enrolled to achieve [redacted] randomized in Part 2.

4.2. Selection of Participants

This study will enroll male and female participants ≥ 18 years of age. Participants will be eligible for enrollment in this study regardless of race/ethnicity.

4.2.1. Participant Inclusion Criteria

1. Age ≥ 18 years.
2. Able and willing to voluntarily complete the informed consent process
3. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin, sepiapterin and/or Phe-restricted diet), demonstrated by uncontrolled blood Phe level > 360 µmol/L on current therapy any time during screening and uncontrolled blood Phe level > 360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the participant's medical history (inclusive of any screening values). All screening values must be obtained more than 7 days apart, as determined by central or local laboratory.

4. Females of childbearing potential must have a negative pregnancy test at screening and the end of DEP (in order to enter Part 2) and RWP (in order to enter Part 3) and be willing to have additional pregnancy tests during the study.

5. Sexually active female participants of childbearing potential must be willing to use an acceptable method of contraception while participating in the study and for 2 weeks after the last dose.

6. Stable diet including stable medical formula regimen (if used) for at least 1 month prior to screening.

7. If using sapropterin or sepiapterin, must be on a stable dose for at least 3 months.

8. Willing and able to continue current diet, sapropterin, sepiapterin and large neutral amino acids unchanged during screening, DEP, and RWP and to engage in all study activities.

4.2.2. Participant Exclusion Criteria

1. Currently taking Palynziq® (pegvaliase-pqpz) (within 1 month of screening).
2. Acute or chronic medical, surgical, psychiatric, or social condition or laboratory abnormality that may increase participant risk associated with study participation, compromise adherence to study procedures and requirements, and, in the judgment of the investigator, would make the participant inappropriate for enrollment.
3. A known or suspected diagnosis of DNAJC12 deficiency, biopterin synthesis deficiency, or irritable bowel syndrome.
4. Intolerance of or allergic reaction to *E. coli* Nissle or any of the ingredients in SYNB1934v1 or placebo formulations, or an allergy to cinnamon. Known intolerance to proton pump inhibitors and H2 blockers, since one or the other must be used.
5. Currently taking or plans to take any type of systemic (e.g., oral or intravenous) antibiotic within 28 days prior to the first dose of IMP through final safety assessment in RWP, including planned surgery, hospitalizations, dental procedures, or interventional studies that are expected to require antibiotics. Exception: topical antibiotics are allowed.
6. Pregnant, planning to become pregnant, or breastfeeding.
7. Current participation in any other investigational drug study or use of any investigational agent within 30 days or 5 half-lives (whichever is longer) prior to screening.
8. Ever received gene therapy for treatment of PKU.

4.3. Treatment of Participants

Within this protocol, IMP refers to SYNB1934v1 or placebo.

4.3.1. SYNB1934

SYNB1934v1 is an engineered bacterium derived from EcN that has been designed to treat PKU by consuming and converting Phe to the metabolites TCA and phenylpyruvate.

SYNB1934v1 IMP consists of powder for oral suspension packaged in sachets. SYNB1934v1 is administered orally so that it acts upon Phe liberated from the diet in the gastrointestinal tract. It is provided 3 times daily with meals so that it is effective in covering protein intake during the course of the day. During dose preparation, the powder will be resuspended in 100 mL of water or apple juice. The prepared dose is to be administered within 15 minutes of resuspension.

During the first 9 days of the DEP, participants will [redacted] before using the full dose of 3×10^{11} cells (see Table 4). This partial amount, only used during ramping, is prepared by making a [redacted].

The study pharmacist or their designee is responsible for tracing the medication. Drug accountability for this study will be maintained through the electronic data capture system on an ongoing basis. All unused IMP must be returned by participants to the site for destruction per local requirements. Detailed instructions for the storage, handling, administration, and disposal of IMP will be provided in the study-specific pharmacy manual and in a participant instruction pamphlet.

4.3.2. Placebo

A placebo will be manufactured using an inactive powder that is color matched to the SYNB1934v1 drug product. In order to maintain study blinding during the RWP, placebo will be packaged, labeled, stored, and administered in an identical manner to SYNB1934v1.

4.3.3. Treatment Compliance

Study participants will prepare the individual doses at home. They will be instructed on the required procedures by trained study personnel and will receive written instructions on IMP preparation and storage. Compliance will be assessed by recording the number of sachets received and used by each participant. Participants who take ≥ 75% of doses will be considered compliant.

4.3.4. Concomitant Medications

Concomitant medications can be administered at the investigator's discretion to conform to standard practice. Given the gut-restricted mechanism of action, SYNB1934v1 has a low likelihood for eliciting clinically relevant drug–drug interactions; however, no traditional drug–drug interaction studies have been conducted. Investigators should use caution when prescribing co-medications and should contact the medical monitor if they are unsure whether a drug should be prescribed to a participant. All concomitant medications and dietary supplements will be recorded in the electronic case report forms (eCRFs), using generic drug names when possible.

4.3.4.1. Proton Pump Inhibitor

During participation in the study, participants will take a PPI [redacted] QD (recommended to occur before breakfast) starting 7 days before first dose of IMP in Part 1. The PPI should be taken at the same time each day, even if no meal is consumed. If participants are already on a PPI regimen consistent with instructions in the package insert, they

may continue on that and not [redacted]. If participants cannot tolerate a PPI, they can use an H2 blocker per investigator discretion. If neither a PPI nor H2 blocker are tolerated during the screening, the participant will discontinue the study. The dose of PPI or H2 blocker should remain stable for Parts 1 and 2 of the study; if a dose adjustment to the PPI or H2 blocker is required in Part 3, the investigator must discuss with the medical monitor (unless the adjustment is required due to an adverse event). If a PPI or H2 dose is missed, it should be taken as soon as realized and then resume the normal before-breakfast QD or BID schedule, according to the locally approved PPI or H2 label.

4.3.4.2. [redacted]

During participation in the study, participants will be provided with o[redacted], an approved medication for [redacted]The use of this medication in the study is optional and at the discretion of the investigator (see Section 3.2.1.1).

4.3.4.3. Antibiotics

Participants who anticipate requiring systemic (e.g., oral or intravenous) antibiotics are not eligible; however, unanticipated antibiotic administration may occur during the study. Topical antibiotics are permitted at any time.

Participants who require systemic antibiotics in Parts 1 and 2 will continue IMP dosing but will not be included in the per-protocol analysis set (see Section 8.2).

Participants who require systemic antibiotics in OLE will continue IMP dosing. Any planned Phe assessments taken during antibiotic treatment or within 14 days of the last dose of antibiotics in the OLE should be obtained > 14 days after the completion of antibiotic treatment.

4.3.4.4. Prohibited Therapies

Palynziq® (pegvaliase-pqpz) is prohibited within 1 month of screening through the duration of the study, as well as any investigational product from screening through the duration of the study.

Participants who have ever received gene therapy for PKU are not eligible for the study.

5. STUDY PROCEDURES AND ASSESSMENTS

5.1. Schedule of Events

Table 5 and Table 6 presents the schedules of events for the main study. A description of the study procedures is provided in the following sections.

Table 5: Schedule of Events: Dose-escalation (Part 1) and Randomized Withdrawal (Part 2)

		DEP (Part 1)			RWP (Part 2)		
Description/Week[a]	**Screen[b]**	**Study/DEP Baseline**	**iTD-Finding Period**	**End of DEP[b] (RWP Baseline)[c]**	**Wk 1**	**Wk 3**	**Wk 4 (OLE Baseline)[c]**
Study Day	**-45 to -1**	**1**	**Up to 105 days[d]**	**21 days after iTD**	**7**	**21**	**28**
Assessment[b] (Protocol Section)							
Informed consent (11.5)	●						
Medical history and prior medications (5.2)	●						
Vital signs (SBP/DBP, pulse, body temperature, height, weight[e]) (5.5.2)	●	●		●			●
Physical examination (5.2)	●						
FSH test (postmenopausal women only) (5.5.3)	●						
Pregnancy test (WOCBP only)[f] (5.5.3)	●	●		●			●
Record concomitant medications (4.3.4)	●	●	●	●	●	●	●
Adverse event reporting (5.5.1)	●	●	●	●	●	●	●
Collection of adverse event diary cards (5.5.1)		●	●	●	●	●	●
Weekly contact by site personnel (telephone, text, email)			●	●	●	●	●
Randomization (8.11)				●			
Blood Phe[g] and Tyr (5.4.1)	●	●	●	●	●	●	●
3-Day dietary intake assessment[h] (3.3)		●	●	●	●	●	●
Administer PPI once per day (4.3.4.1)	●[i]	●	●	●	●	●	●
IMP immediately after meals[j] (3.2)		●	●	●	●	●	●
Laboratory tests (hematology/CBC with differential, CRP, serum chemistry, urinalysis) (5.5.3)	●	●		●			●
Electrocardiogram (supine for 5 minutes)(5.5.2)	●						
Immunogenicity sample[k] (5.2)		●					
Optional fecal sample for microbiome analysis[l] (5.2)	●						

AE = adverse event; CBC = complete blood count; CRP = C-reactive protein; DEP = dose-escalating period; DBP = diastolic blood pressure; FSH = follicle-stimulating hormone; IMP = investigational medicinal product; iTD = individually titrated dose; Phe = phenylalanine; PPI = proton pump inhibitor; RWP = randomized withdrawal period; SBP = systolic blood pressure; Term = termination; Tyr = tyrosine; WOCBP = women of childbearing potential.

a Weeks are relative to the study period (i.e., each part begins with Week 1). All assessments/visits may be conducted within a 3-day window.

b All visits/assessments may be performed in the clinic or by a home healthcare professional at an alternate location (e.g., home, office, etc.), except for screening and end of DEP which must either be in person or via a virtual platform capable of completing all scheduled events including examination.

c Participants who discontinue prematurely during the DEP will complete an early termination visit 30 days after the last dose of IMP as described in Table 6. Participants who discontinue prematurely during RWP will complete RWP study measures as scheduled prior to the early termination visit. Early termination visits are described in Section 5.6.

d Participants may titrate down to the previous dose level during the iTD period if there is difficulty tolerating a higher dose level or at the investigator's discretion. Visits and blood draws occur after a participant has been at a dose level for 3 weeks (including the ramp of that dose level as shown in Table 4). Participants must remain on their iTD for 3 weeks before proceeding to Part 2. Participants cannot up-titrate again until the OLE.
e Height and weight measured only at screening.
f Serum pregnancy test at screening; urine pregnancy test at other visits.
g All blood Phe should be drawn in duplicate. Baseline samples in DEP and RWP must be taken prior to the first doses in those periods. In Part 1, blood Phe will be measured every 3 weeks after a change in dose level during the iTD-finding period. In Part 2, blood Phe will be measured at Weeks 1, 3, and 4. See Section 5.4.1 of the protocol for more information. Patients enrolled in the optional DEP weekly substudy will have weekly [redacted] See Section 5.4.2 of the protocol for more information.
h A 3-day dietary intake assessment will occur for 3 consecutive days prior to scheduled study visits and blood Phe draws. See Diet Manual for more information.
i The first dose of a PPI will be taken on or before Day –7 and continue daily as described in Section 4.3.4.1.
j Investigational medicinal product should be taken within 30 minutes of finishing a meal. See the Diet Manual for further details.
k Samples will be used for monitoring anti-drug antibodies as needed (see Section 6.3).
l Fecal samples can be done at any time during the screening period, no less than 5 days prior to baseline visit.

Table 6: Schedule of Events: Part 3, Open-Label Extension Period and Follow-up, End of Study and Early Termination Visits

	Quarterly Visits	End of Study	Early Term.
	Every 90 days ± 5 days	at least 30 days after last dose	at least 30 days after last dose
Assessment[a] (Protocol Section)			
Vital signs (SBP/DBP, pulse, body temperature) (5.5.2)	●	●	
Pregnancy test (WOCBP only)[b] (5.5.3)	●	●	
Record concomitant medications (4.3.4)	●	●	●
Adverse event reporting (5.5.1)	●	●	●
Blood Phe[c] and Tyr (5.4.1)	●		
3-Day dietary intake assessment[d] (3.3)	●		
Administer PPI once per day (4.3.4.1)	●		
IMP immediately after meals (TID) (Section 3.2.3)	●		
Laboratory tests (hematology/CBC w/differential, CRP, serum chemistry) (5.5.3)	●	●	
Optional fecal sample for microbiome analysis (5.2)		●	

AE = adverse event; CBC = complete blood count; CRP = C-reactive protein; DEP = dose-escalating period; DBP = diastolic blood pressure; IMP = investigational medicinal product; Phe = phenylalanine; PPI = proton pump inhibitor; RWP = randomized withdrawal period; SBP = systolic blood pressure; Term = termination; TID = 3 times per day; Tyr = tyrosine; WOCBP = women of childbearing potential.
a All visits/assessments may be performed in the clinic or by a home healthcare professional at an alternate location (e.g., home, office).
b Urine pregnancy test at quarterly visits.
c All blood Phe should be drawn in duplicate.
d A 3-day dietary intake assessment will occur immediately before all scheduled study visits.

5.2. Screening and Baseline Periods

Screening procedures must be performed to determine eligibility for enrollment within 45 days before the first dose of IMP. Participants who fail screening due to a transient cause or missing information are eligible for rescreening once, with a minimum of 7 days between screenings. During screening, a unique number will be assigned to each participant who signs an informed consent form (ICF).

Participants meeting all the inclusion criteria and none of the exclusion criteria who are selected to participate in the study will be enrolled and begin the DEP baseline period. Once participants are enrolled in the study, they will only be identified by participant number.

All assessments to be performed at screening and baseline are outlined in the Schedule of Events (Table 5). A physical examination will be performed at screening only and can be performed using a virtual platform. Medical history and medications taken over the past 30 days will be recorded at screening. Any ongoing condition as well as signs and symptoms observed prior to the participant signing the ICF should be recorded as medical history.

An optional fecal sample will be collected at screening and end of study for microbiome analysis. Blood samples will also be collected to aid in the investigation of any immunological reaction seen during the study.

5.3. Randomization

At the end of the DEP, after completing 3 weeks of dosing at their iTD, participants will be randomized 1:1 to receive either SYNB1934v1 at their iTD established in the DEP or placebo, stratified by the qualifying blood Phe value from screening. This screening value will be the value determined by the central lab, except for participants where the local screening value was > 360 µmol/L and the central screening value was ≤ 360 µmol/L, in which case the local screening value will be used for stratification. Stratification will be based upon screening blood Phe levels of 361 to 720 µmol/L or > 720 µmol/L. This stratification has been selected to dichotomize the participant population by control, based upon prior surveys of patients with PKU.

5.4. Pharmacodynamic Assessments

5.4.1. Blood Phenylalanine and Tyrosine

Blood Phe and Tyr samples will be drawn [redacted] will be drawn in duplicate, using separate tubes from the same venipuncture, and analyzed separately. The time of the last meal will be recorded when taking Phe samples.

Baseline samples for DEP and RWP must be drawn prior to the first dose for those respective periods. During the DEP, blood Phe and Tyr will be measured at Week 3 of each completed dose level (Table 5). Blood Phe and Tyr will be measured at Weeks 1, 3, and 4 during the RWP (Table 5). During the OLE, blood Phe and Tyr will be measured quarterly (Table 6).

5.4.2. DEP Weekly Substudy

Participants will have the option to enroll in a substudy [redacted] at each dose level. Incentives will be provided to ensure that at least twenty patients are recruited into this substudy. Participants in this substudy [redacted]

5.5. Safety Assessments

5.5.1. Adverse Events

Adverse events will be assessed continuously by direct observation and participant interviews. For GI AEs, participants will also fill out a diary card during screening, DEP, and RWP to allow for enhanced capture of these AEs. The diary cards will be collected at each scheduled visit, excluding the initial screening visit and visits associated with the weekly substudy. The severity of AEs will generally be evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), v5.0, with exceptions described in Section 6.1.1.1. All AEs occurring from the time a participant signs the ICF through the follow-up period will be recorded, regardless of causal assessment to IMP.

5.5.2. Vital Signs, Weight, Height, and Electrocardiograms

Semi-supine vital signs (systolic blood pressure, diastolic blood pressure, pulse, and body temperature), weight, and height will be collected as specified in Table 5 and Table 6. Participants are required to remain in the semi-supine position for at least 5 minutes prior to obtaining vital signs.

Supine single 12-lead electrocardiograms (ECGs) will be performed at screening as specified in Table 5.

5.5.3. Clinical Laboratory Measurements

Clinical safety laboratory tests will be performed at the time points specified in Table 5 and Table 6: chemistry panel, CBC with differential, and creatinine clearance. A pregnancy test for WOCBP and an FSH test for postmenopausal women will be performed at the time points specified in the Schedule of Events. See Appendix 1 for a list of clinical laboratory tests.

Screening results will be assessed by the investigator for inclusion of participants in the study. Additionally, unscheduled clinical laboratory tests may be obtained at any time during the study at the investigator's discretion. The diagnosis corresponding to any clinically significant abnormality or abnormality requiring treatment/intervention must be recorded as an AE.

If genetic testing for PAH mutations was performed before screening, these data may be submitted at the time of screening. Genetic testing is not required for entry into the study, and no sample will be drawn for DNA analysis.

5.6. Early Termination Assessments

Participants who discontinue prematurely during the DEP or OLE will complete an early termination visit no sooner than 30 days after the last dose of IMP, as detailed in Table 6. Participants who discontinue prematurely during the RWP will complete RWP visits as scheduled prior to the early termination visit and will also complete an early termination assessment no sooner than 30 days after the last dose of IMP. Participants will be discharged from the study after this visit.

5.7. End of Study Assessment

Participants who complete the OLE will have an end of study visit no sooner than 30 days after the last dose of IMP, as detailed in Table 6. The end of study visit may include a fecal sample for microbiome analysis.

6. ADVERSE EVENTS

6.1. Adverse Event Definition

An AE is any untoward medical occurrence, including the exacerbation of a preexisting condition, in a participant administered a pharmaceutical product, regardless of causality.

6.1.1. Assessment of Severity

The severity rating of an AE refers to its intensity. The severity of each non-GI related TEAE will be categorized using the CTCAE. For any term that is not specifically listed in the CTCAE scale, intensity should be assigned a grade of 1 through 5 using the following CTCAE guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

6.1.1.1. Gastrointestinal-related Events

Participants will be asked to fill out diary cards with information about GI AEs during screening, DEP, and RWP. The diary cards will be collected at each scheduled visit, excluding the initial screening visit and visits associated with the weekly substudy. In addition, exceptions to CTCAE will be implemented for grading certain GI-related TEAEs, as detailed in Table 7.

Table 7: Severity Assessment of Diarrhea and Nausea

Grade	Diarrhea[a]	Nausea
1	Increase of diarrhea, < 3 liquid stools a day over baseline; mild increase in ostomy output compared to baseline	Loss of appetite without alteration in eating habits and/or with use of planned anti-nausea medication
2	Diarrhea ≥ 3 liquid stools daily and limiting instrumental ADLs	Use of unplanned concomitant medications to increase oral intake
3	Diarrhea requiring hospitalization or limiting self-care ADLs	Inadequate oral caloric or fluid intake; tube feeding, TPN or hospitalization indicated (unchanged from CTCAE)
4	Life-threatening consequences; urgent intervention indicated (unchanged from CTCAE)	Not applicable
5	Death (unchanged from CTCAE)	Not applicable

ADLs = activities of daily living; CTCAE = Common Terminology Criteria for Adverse Events.

[a] Diarrhea is defined as liquid stools without consistency in shape.

6.1.2. Assessment of Causality

Medical judgment should be used to determine the cause of the AE, considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease,

concomitant medication, relevant history, pattern of the AE, temporal relationship to IMP or PPI, and dechallenge or rechallenge.

Related: there is a reasonable possibility that IMP or PPI caused the event; 1 or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of IMP or PPI.
- The event could not be reasonably attributed to the known characteristics of the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant.
- The event follows a known pattern of response to SYNB1934v1 or PPI.
- The event disappears or decreases on cessation or reduction in dose. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study dosing despite other clear indications of relatedness.)

Unrelated: there is no reasonable possibility that the IMP or PPI caused the event; 1 or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of IMP or PPI.
- The event could be reasonably attributed to the known characteristics of the participant's clinical state, concurrent illness, environmental or toxic factors, or other modes of therapy administered to the participant.
- The event does not follow a known pattern of response to SYNB1934v1 or PPI.
- The event does not disappear or decrease on cessation or reduction in dose, and it does not reappear or worsen when dosing is resumed.

6.2. Serious Adverse Events

An SAE is any untoward medical occurrence that meets any of the following criteria:

- Results in death.
- Is immediately life-threatening (refers to an event in which the participant is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Based on appropriate medical judgment, represents an important medical event that may jeopardize the participant or may require intervention to prevent 1 of the other outcomes described above.

6.2.1. Clarification of Serious Adverse Event Definition

- Death is an outcome of an SAE and not an SAE in itself. When death is an outcome, the event(s) resulting in death should be reported (e.g., "pulmonary embolism" with a fatal outcome). The appropriate diagnosis or term should be recorded and assigned severity Grade 5.
- In instances of death due ultimately to the underlying disease, the cause of death should be indicated as the specific event or condition resulting in death to the extent possible. If no appropriate term with a Grade 5 severity in the CTCAE can be identified, then a term should be selected from the CTCAE category "death."
- "Life-threatening" means that the participant was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity. Grade 4 events (e.g., thrombocytopenia) are not always serious unless they have life-threatening consequences or result in hospitalization.
- Preplanned or elective hospitalizations, including social and/or convenience situations (e.g., respite care), are excluded from SAE reporting. In addition, "admissions" under 23-hour Observation or Emergency Room visits are excluded from SAE reporting; however, such events should still be reported on the appropriate eCRF page.
- Overdose of either SYNB1934v1 (defined as a total dose $\geq 2 \times 10^{13}$ live cells in a 24-hour period) or concomitant medication without any overdose signs or symptoms unless the event meets SAE criteria (e.g., hospitalization) are excluded from SAE reporting; however, such events should still be reported on the appropriate eCRF page.
- SAEs that occur within the safety follow-up period but are related to subsequent therapies are excluded from SAE reporting:
    - Participants who have completed study dosing or who terminate from study and then undergo subsequent therapies during the safety follow-up period and experience an SAE specifically related to the administration of the subsequent therapy will not have those events reported as SAEs. This exclusion will include "elective" hospitalizations necessary for the administration of such therapies.

6.2.2. Serious, Unexpected, Suspected Adverse Reactions

In accordance with regulatory requirements, the sponsor or designee will immediately notify regulatory authorities and the investigators, who will in turn notify their IRB/REB as necessary, of any AE associated with IMP administration or study procedures that is a serious, unexpected, suspected adverse reaction or any finding from tests in laboratory animals that suggests a significant risk for human participants, including reports of mutagenicity, teratogenicity, or carcinogenicity. An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been previously observed.

6.3. Adverse Events of Special Interest

An adverse event of special interest (AESI), whether serious or nonserious, is one of scientific and medical concern specific to SYNB1934v1 for which ongoing monitoring and rapid communication by the investigator may be appropriate. Based on current preclinical and clinical data, the following AEs will be considered AESIs and should be reported to the Sponsor within 24 hours of awareness.

Adverse events of special interest for SYNB1934v1 are as follows:

- Hypersensitivity reactions due to SYNB1934v1.
- Infections due to SYNB1934v1.
- Grade ≥ 3 gastrointestinal AEs per protocol specified grading criteria, including nausea, vomiting, diarrhea, and abdominal pain.

6.4. Reporting of Adverse Events

All AEs, serious and nonserious, will be fully documented on the appropriate eCRF. For each AE, the investigator must provide its duration (start and end dates or ongoing), intensity, assessment of causality, and whether specific action or therapy was required.

All AEs occurring from the time a participant signs the ICF through the safety follow-up period and at least one month following the last dose of SYNB1934v1 must be recorded on the eCRF.

All AESIs and SAEs, regardless of relationship to SYNB1934v1 or [redacted], must be reported to the Sponsor/designee on the applicable eCRF form within 24 hours of awareness. In addition, AESI or SAE forms should be uploaded and submitted to Synlogic.safety@arriello.com within 24 hours of awareness. All AESIs and SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. After the final follow-up visit, only IMP-related AEs/SAEs need to be collected and reported.

6.5. Pregnancy

Women of childbearing potential must have a negative serum pregnancy test at screening. A urine pregnancy test will be taken at timepoints outlined in the Schedule of Events (Table 5 and Table 6) and must be negative. Women of childbearing potential must agree to use acceptable methods of contraception, after informed consent, throughout the study, and for a minimum of 2 weeks after dosing has been completed. Breastfeeding women or those planning to become pregnant are excluded from the study.

If a participant becomes pregnant during the study, treatment must be discontinued. The pregnancy must be reported to the Sponsor/designee and the IRB/IEC/REB. The applicable eCRF and pregnancy report form should be completed within 24 hours of awareness by clinical site staff. Pregnancy report forms should be sent to Synlogic.safety@arriello.com. Follow-up evaluation of the pregnancy, fetus, and child should be performed. If the participant chooses to continue the pregnancy, then her pregnancy should be followed to term and 3 months after delivery, so that any important safety information can be obtained.

Males participating in the study do not need to take specific precautions to prevent pregnancy.

6.6. Clinical Laboratory Abnormalities

It is the responsibility of the investigator to assess the clinical significance of all abnormal laboratory values as defined by the appropriate reference ranges. All abnormal values assessed to be of clinical concern and at least possibly related to IMP or of uncertain causality should be repeated. Persistent abnormal values and changes of possible clinical concern that remain within the normal range should be followed at the discretion of the investigator.

An abnormal laboratory value that is not already associated with an AE is to be recorded as an AE if an action on IMP dosing is made as a result of the abnormality, if intervention for management of the abnormality is required, or at the discretion of the investigator.

6.7. Review of Safety Data

The DMC, investigator, and sponsor medical monitor will be responsible for the ongoing review and evaluation of safety data, including AEs, clinical laboratory data, and any other safety evaluations, for the duration of the study. Details will be provided in the DMC charter.

The first DMC meeting will occur when [redacted] have completed the DEP or when the [redacted] beyond the DEP baseline visit, whichever occurs first.

The DMC will also meet after the [redacted] participants have completed the DEP and when the last participant has completed the RWP.

All meetings will be used to review safety, to ensure that there is an adequate responder rate for the RWP primary analysis population, and to evaluate the DEP baseline Phe values of the population prior to treatment. The sponsor will remain blinded to the subject-level blood-Phe levels; however, the DEP overall responder rates and DEP baseline Phe values as well as the interim analysis results will be provided to the sponsor by the DMC.

7. ESTIMANDS

The following are the descriptions of the estimands for the primary and key secondary endpoints in the study, based on the methods described in ICH-E9 (R1).

7.1. Part 1: Primary and Key Secondary Efficacy Objective Estimands

The estimands corresponding to the Part 1 DEP primary and key secondary objectives and endpoints are described in Table 8 and Table 9, respectively.

Table 8: Estimand for the Part 1 Primary Objective

Objective	To assess the percentage change in blood Phe level
A. Population	The population is the DEP full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Continuous variable: Percent change from DEP baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1

C. Intercurrent events	• Participant does not reach an iTD or iTD dose is dropped: As described in Section 3.1, the participant's iTD will be the highest dose tolerated. Additionally, as described in Section 8.12, doses may be dropped at the DEP interim analysis. A principal stratum approach will be taken in that the target population is the set of participants who reach an iTD and the corresponding dose is not dropped at the interim analysis. • Treatment discontinuation: If a participant permanently discontinues study drug in the DEP, the participant will be asked to have an early termination visit 30 days after last dose of study drug (see Section 3.5). Treatment discontinuation will follow the principal stratum approach in that if the participant has reached an iTD (defined as having reached an iTD if they tolerate 3 weeks at a dose, regardless of whether other doses are tolerated) the participant will be included in the analysis. • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered (i.e., all observed data will be used regardless of antibiotic use). • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered (i.e., all observed data will be used regardless of prohibited medication use).
D. Population-level summary	The population-level summary is the least-squares mean for the percent change from baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1 derived from an MMRM using an observed case approach. The MMRM model is described in Section 8.4.1.

DEP = dose-escalating, open-label period; iTD = individually titrated dose; MMRM = mixed model with repeated measures; Phe = phenylalanine.

Table 9: Estimands for the Part 1 Key Secondary Objectives

Objective	To assess the absolute change in Phe level
A. Population	The population is the DEP full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Continuous variable: Change from DEP baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1
C. Intercurrent events	• Participant does not reach an iTD or iTD dose is dropped: As described in Section 3.1, the participant's iTD will be the highest dose tolerated. Additionally, as described in Section 8.12, doses may be dropped at the DEP interim analysis. A principal stratum approach will be taken in that the target population is the set of participants who reach an iTD and the corresponding dose is not dropped at the interim analysis. • Treatment discontinuation: If a participant permanently discontinues study drug in the DEP, the participant will be asked to have an early termination visit 30 days after last dose of study drug (see Section 3.5). Treatment discontinuation will follow the principal stratum approach in that if the participant has reached an iTD (defined as having reached an iTD if they tolerate 3 weeks at a dose, regardless of whether other doses are tolerated) the participant will be included in the analysis • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered (i.e., all observed data will be used regardless of antibiotic use). • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered (i.e., all observed data will be used regardless of prohibited medication use).
D. Population-level summary	The population-level summary is the least-squares mean for the change from baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1 derived from

	an MMRM using an observed case approach. The MMRM model is described in Section 8.5.1.
Objective	To determine the responder population
A. Population	The population is the DEP full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Dichotomous variable: A ≥ 20% reduction from baseline in blood Phe level at any time in the DEP
C. Intercurrent events	• Participant does not reach an iTD or iTD dose is dropped: As described in Section 3.1, the participant's iTD will be the highest dose tolerated. Additionally, as described in Section 8.12, doses may be dropped at the DEP interim analysis. A principal stratum approach will be taken in that the target population is the set of participants who reach an iTD and the corresponding dose is not dropped at the interim analysis. • Treatment discontinuation: If a participant permanently discontinues study drug in the DEP, the participant will be asked to have an early termination visit 30 days after last dose of study drug (see Section 3.5). Treatment discontinuation will follow the principal stratum approach in that if the participant has reached an iTD (defined as having reached an iTD if they tolerate 3 weeks at a dose, regardless of whether other doses are tolerated) the participant will be included in the analysis • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered (i.e., all observed data will be used regardless of antibiotic use). • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered (i.e., all observed data will be used regardless of prohibited medication use).
D. Population-level summary	The proportion of participants meeting the ≥ 20% reduction from baseline in blood Phe level at any time in the DEP will be summarized along with 95% confidence intervals.

DEP = dose-escalating, open-label period; iTD = individually titrated dose; MMRM = mixed model with repeated measures; Phe = phenylalanine.

7.2. Part 2: Primary and Key Secondary Efficacy Objectives Estimands

The estimands corresponding to the Part 2 RWP primary and key secondary objectives and endpoints are described in Table 10 and Table 11, respectively.

Table 10: Estimand for the Part 2 Primary Objective

Objective	To evaluate efficacy of SYNB1934v1 versus placebo in the responder population
A. Population	The population is the RWP responder full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Continuous variable: Change from RWP baseline to Week 4 in blood Phe level
C. Intercurrent events	• Treatment discontinuation: consistent with a treatment-policy strategy, treatment discontinuation will not be considered. • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered. • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered.

Objective	To evaluate efficacy of SYNB1934v1 versus placebo in the responder population
D. Population-level summary	The population-level summary is the least-squares means and difference in the change from baseline to Week 4 in blood Phe levels between the SYNB1934v1 and placebo treatment groups. An MMRM using an observed case approach is being applied. The post-baseline blood Phe levels at Weeks 1, 3, and 4 will be used. The MMRM model is described in Section 8.4.2.

MMRM = mixed model with repeated measures; Phe = phenylalanine; RWP = randomized withdrawal period.

Table 11: Estimands for the Part 2 Key Secondary Objectives

Objective	To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to the proportion of participants with a blood Phe level ≤ 360 µmol/L
A. Population	The population is the RWP responder full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Dichotomous variable: blood Phe level ≤ 360 µmol/mL at Week 4
C. Intercurrent events	• Treatment discontinuation: consistent with a treatment-policy strategy, treatment discontinuation will not be considered. • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered. • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered.
D. Population-level summary	Difference in proportions between the SYNB1934v1 and placebo treatment groups. The analysis method is described in Section 8.5.
Objective	To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to the change from DEP baseline in blood Phe level
A. Population	The population is the RWP responder full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Continuous variable: Change from DEP baseline to Week 4 in blood Phe level.
C. Intercurrent events	• Treatment discontinuation: consistent with a treatment-policy strategy, treatment discontinuation will not be considered. • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered. • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered.
D. Population-level summary	The population-level summary is the least-squares means and difference in the change from DEP baseline to Week 4 in blood Phe levels between the SYNB1934v1 and placebo treatment groups. An MMRM using an observed case approach is being applied. The post-baseline blood Phe levels at Weeks 1, 3, and 4 will be used. The MMRM model is described in Section 8.5.
Objective	To evaluate the efficacy of SYNB1934v1 versus placebo in the responder population with regard to the percent change from DEP baseline in blood Phe level
A. Population	The population is the RWP responder full analysis set (defined in Section 8.2) characterized by the inclusion/exclusion criteria provided in Section 4.2.
B. Variable	Continuous variable: Percent change from DEP baseline to Week 4 in blood Phe level.

C. Intercurrent events	• Treatment discontinuation: consistent with a treatment-policy strategy, treatment discontinuation will not be considered. • Antibiotic use: consistent with a treatment-policy strategy, antibiotic use will not be considered. • Prohibited medication use: consistent with a treatment-policy strategy, prohibited medication use will not be considered.
D. Population-level summary	The population-level summary is the least-squares means and difference in percent change from DEP baseline to Week 4 in blood Phe levels between the SYNB1934v1 and placebo treatment groups. An MMRM using an observed case approach is being applied. The post-baseline blood Phe levels at Weeks 1, 3, and 4 will be used. The MMRM model is described in Section 8.5.

DEP = dose-escalating, open-label period; MMRM = mixed model with repeated measures; Phe = phenylalanine; RWP = randomized withdrawal period.

8. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

8.1. General Statistical Methods

All evaluations and tabulations will be carried out as described in detail in a statistical analysis plan (SAP), which will be finalized and approved prior to the DEP interim analysis and database lock and unblinding.

A disposition of participants will be provided for each of the 3 parts of the study and will include a breakdown of participants who were randomized, treated, discontinued treatment, lost to follow-up, or withdrew consent (as appropriate).

Continuous variables will be summarized using the number of observations, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages.

Baseline for blood Phe level in each of the 3 parts of the study will be defined as the mean of the duplicate blood Phe level measurements obtained immediately prior to administration of the first dose of each part. If only one blood Phe level measurement is available, then that measure will be used as baseline. Similarly, for the determination of responders and participants who achieve a specific reduction in Phe (e.g., Phe level ≤ 360 µmol/L at any time in the DEP, or a ≥ 20% reduction from baseline) the mean of the duplicate blood Phe level measurements at the applicable post-baseline timepoint (e.g., Week 3) will be used. If only one of the duplicate blood Phe levels is available at a particular post-baseline timepoint, then that measurement will be used. In describing the endpoints, the baseline and week numbering are specific to the corresponding study part, unless explicitly noted otherwise.

8.2. Populations for Analysis

Analysis populations will be defined for each of the 3 parts of the study (DEP, RWP, and OLE).

Full analysis set (FAS): The full analysis sets are based on the intention-to-treat principle. The DEP FAS includes all participants enrolled in the DEP.

The RWP FAS is defined as all participants randomized into the RWP. Additionally:

- The responder RWP FAS is defined as participants in the RWP FAS who are responders
- The nonresponder RWP FAS is defined as participants in the RWP FAS who are nonresponders

A responder is defined as a participant who achieves a ≥ 20% reduction in blood Phe level compared to DEP baseline on SYNB1934v1 (i.e., this is an exploratory endpoint for the DEP, see Table 1).

The responder RWP FAS will be the primary efficacy analysis population for the RWP. Analyses on the RWP FAS will be performed according to the randomized treatment group.

In the event that, based on the interim analysis results utilizing the criteria provided in Table 12, the DMC recommends dropping either the 1×10^{12} or the 1×10^{12} and 6×10^{11} doses, the DEP FAS will exclude all participants who were enrolled and treated at a dropped dose in the DEP. Additionally, the RWP FAS will exclude all participants who have been randomized in the RWP up to this time. The participants in the DEP who have an iTD corresponding to a dropped dose will go directly into the OLE part of the study (i.e., as opposed to being randomized into the RWP).

These participants who are excluded from the DEP FAS and RWP FAS will be summarized separately.

Safety analysis set (SAS): For each of the 3 study parts, the SAS will include all participants who received any amount of IMP in the corresponding study part with treatment assignment based on the treatment received.

Per-protocol analysis set (PPS): The DEP PPS is defined as all DEP FAS participants, who had no major protocol deviations that would affect efficacy, completed their final 3-week iTD, whose dietary Phe remained within 20% of their DEP baseline value, and who did not require systemic antibiotic treatment during DEP. The DEP PPS will be used as a sensitivity analysis for the DEP primary efficacy endpoint.

The responder RWP PPS is defined as all responder RWP FAS participants, who had no major protocol deviations that would affect efficacy, had at least 75% treatment compliance, whose dietary Phe remained within 20% of their DEP baseline value, and who did not require systemic antibiotic treatment during DEP or RWP (see Section 4.3.4.2). The responder RWP PPS will be used as a sensitivity analysis for the RWP primary efficacy endpoint. Major protocol deviations will be determined prior to the RWP database lock. Analyses on the responder RWP PPS will be performed according to the randomized treatment group.

8.3. Determination of Sample Size

[redacted]. This assumes a dropout rate of [redacted] and approximately 50% of participants are responders. If, during the course of the study, it becomes apparent that the assumptions were not accurate or a dose level is dropped after the interim analysis, the number of participants enrolled into the DEP may be adjusted. The study has over

[redacted]. The mean percent reduction and standard deviation in blood Phe values used in the power calculations are based on results from a similar study in participants with PKU.[26]

A sample size of [redacted], a [redacted] The power was calculated via a t-test. The mean treatment group difference and standard deviation in blood Phe values used in the power calculations are based on results from a similar study in participants with PKU.[26]

8.4. Primary Efficacy Endpoint Analyses

The primary efficacy analyses for DEP and RWP are presented in the sections below. Sensitivity and supplemental analyses to support these analyses will be presented in the SAP.

8.4.1. Part 1: DEP

The DEP primary efficacy endpoint is the percent change from DEP baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1. The last measurement is the participant's last Week 3 blood Phe level at the iTD of SYNB1934v1 (ie, as discussed in Section 3.2.1).

The mean percent change from baseline at the participant's iTD will be tested by an MMRM analysis. The MMRM model will have DEP baseline blood Phe level and iTD dose level as fixed effects. The dependent variable is the percent change from baseline in blood Phe level at the last measurement of the iTD of SYNB1934v1.

Least-squares means for the percent change from baseline overall and at each iTD dose level, along with the corresponding [redacted] and p-values testing a percent change from baseline equal to 0, will be calculated. The primary efficacy analysis will test the least-squares means percent change from baseline equal to 0 for the blood Phe level at the participants' last week (Week 3) at his or her established iTD.

At each of the scheduled assessments blood Phe will be drawn in duplicate. The MMRM will incorporate these participant repeated measures. For the MMRM analysis, the results of the post-baseline duplicate samples from the Week 3 visit will be included in the statistical model (i.e., without averaging the results within a given visit before inclusion into the MMRM).

The DEP FAS will be the primary efficacy analysis population.

8.4.2. Part 2: RWP

The RWP primary efficacy endpoint is the change from RWP baseline to Week 4 in blood Phe level. An MMRM analysis will be used to compare the mean change in blood Phe level between the placebo and SYNB1934v1 dose groups. The MMRM model will have treatment group, RWP

baseline blood Phe level, iTD dose level, visit, and visit × treatment group (interaction effect), visit × iTD dose level (interaction effect), and visit × RWP baseline blood Phe level (interaction effect) as fixed effects. The MMRM will also incorporate the participant's repeated measures at visits Weeks 1, 3, and 4, with visit being treated as a categorical variable. Least-squares means for each treatment group and the SYNB1934v1 treatment group difference from placebo, along with the corresponding [redacted] will be calculated for Weeks 1, 3, and 4. The primary efficacy analysis will compare the least-squares means treatment difference between SYNB1934v1 and placebo in the change from baseline in the blood Phe level at Week 4.

At each of the scheduled assessments, blood Phe will be drawn in duplicate. The MMRM will incorporate these participant repeated measures. For the MMRM analysis, the results of each of the post-baseline duplicate samples from a visit will be included in the statistical model (i.e., without averaging the results within a given visit before inclusion into the MMRM).

The responder RWP FAS will be the primary efficacy analysis population.

8.5. Key Secondary Efficacy Endpoint Analyses

8.5.1. Part 1: DEP

The key secondary efficacy endpoints for the DEP are:

- Change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1
- A ≥ 20% reduction from baseline in blood Phe level at any time of the iTD of SYNB1934v1
- A ≥ 20% reduction from baseline in blood Phe level at any time in the DEP.

The key secondary endpoint, change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1, will be analyzed using an MMRM analysis similar to the model described for the DEP primary efficacy analysis (Section 8.4), with the dependent variable being the change in blood Phe level from DEP baseline.

For the 2 key secondary endpoints, a ≥ 20% reduction from baseline in blood Phe level at any time of the iTD of SYNB1934v1, and a ≥ 20% reduction from baseline in blood Phe level at any time in the DEP. The proportion of participants meeting these criteria will be summarized along with 95% confidence intervals.

These analyses will be performed on the DEP FAS.

8.5.2. Part 2: RWP

The key secondary efficacy endpoints for the RWP are:

- Percent change from DEP baseline in blood Phe level at Week 4.
- Change from DEP baseline to Week 4 in blood Phe level
- Blood Phe level ≤ 360 µmol/L at Week 4.

The proportion of participants meeting the key secondary endpoint, a blood Phe level ≤ 360 µmol/L at Week 4, will be tested between the placebo and SYNB1934v1 dose groups using a Fisher's Exact test. The key secondary endpoint, change from DEP baseline to Week 4 in blood Phe level, will be analyzed using an MMRM analysis similar to the model described for the RWP primary efficacy analysis (Section 8.4), with the dependent variable being the change in blood Phe level from DEP baseline, and the DEP baseline blood Phe level as the baseline blood Phe level covariate. The key secondary endpoint, percent change from DEP baseline in blood Phe level at Week 4, will be analyzed using an MMRM analysis similar to the model described for the RWP primary efficacy analysis (Section 8.4), with the dependent variable being the percent change in blood Phe level from DEP baseline, and the DEP baseline blood Phe level as the baseline blood Phe level covariate.

These analyses will be performed on the responder RWP FAS.

8.6. Other Secondary Efficacy Endpoint Analyses

8.6.1. Part 1: DEP

The secondary efficacy endpoints for the DEP are:

- Blood Phe level ≤ 360 µmol/L at any time of the iTD of SYNB1934v1
- Blood Phe level ≤ 360 µmol/L at any time in the DEP.

For each of these 2 endpoints, the proportion of participants meeting the endpoint will be summarized along with [redacted]. These analyses will be performed on the DEP FAS.

8.6.2. Part 2: RWP

The additional secondary efficacy endpoints for the RWP are:

- A ≥ 20% reduction from DEP baseline in blood Phe level at Week 4.

The proportion of participants achieving a ≥ 20% reduction in blood Phe level from baseline to Week 4 will be tested between the placebo and SYNB1934v1 dose groups using a Fisher's Exact test.

These analyses will be performed on the responder RWP FAS.

8.7. Exploratory Endpoint Analyses

The analysis methods for the exploratory endpoints will be presented in the SAP.

8.8. Missing Data

The primary efficacy endpoint analyses for the DEP and RWP will be an observed-case analysis. Missing data will not be imputed for the primary efficacy analyses.

For the following key secondary efficacy analyses, an observed-case analysis will also be applied:

- DEP Part 1: Change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1
- RWP Part 2: Change from DEP baseline to Week 4 in blood Phe level
- RWP Part 2: Percent change from DEP baseline in blood Phe level at Week 4

For the following dichotomous key secondary efficacy analyses, an observed-case approach is being applied such that if a participant is missing the data needed to determine the endpoint, they will be considered as not having met the endpoint.

- DEP Part 1: A ≥ 20% reduction from baseline in blood Phe level at any time of the iTD of SYNB1934v1
- DEP Part 1: A ≥ 20% reduction from baseline in blood Phe level at any time in the DEP
- RWP Part 2: Blood Phe level ≤ 360 µmol/L at Week 4.

Sensitivity analyses considering different methods of handling missing data for the primary and key secondary efficacy endpoints will be specified in the SAP. Additional details on the handling of missing data will be provided in the SAP.

8.9. Multiplicity

The overall Type I error rate for the DEP will be controlled at the 2-sided 0.05 level for the primary endpoint. If the DEP primary endpoint analysis is significant at the 2-sided 0.05 level the key secondary endpoint, change from baseline in blood Phe level at last measurement of the iTD of SYNB1934v1, will be tested at the 2-sided 0.05 level. Note that the 2 dichotomous DEP key secondary endpoints; a ≥ 20% reduction from baseline in blood Phe level at any time of the iTD of SYNB1934v1, and a ≥ 20% reduction from baseline in blood Phe level at any time in the DEP - do not have an associated inferential test.

The overall Type I error rate for the RWP will also be controlled at the 2-sided 0.05 level for the primary endpoint (i.e., DEP, and RWP, will each have a 2-sided Type I error rate of 0.05). The control of multiplicity at 0.05 within each study part is consistent with this study having 3 parts, with each study part having a distinct set of objectives, endpoints, and analysis populations.

For the RWP, the 3 key secondary endpoints will be tested at the 2-sided 0.05 level using a fixed sequential testing methodology in this order:

1) Percent change from DEP baseline to Week 4 in blood Phe level

2) Change from DEP baseline to Week 4 in blood Phe level

3) Blood Phe level ≤ 360 µmol/mL at Week 4

These key secondary endpoints will be tested following this prespecified order only if the RWP primary efficacy endpoint null hypothesis is rejected.

8.10. Safety Analysis

Safety will be evaluated by scheduled monitoring of AEs, vital signs, and clinical laboratory measurements. Safety parameters will be summarized descriptively by the treatment regimen (dose and frequency) that the participant was on at the time of the safety measurement. By-participant listings of all measurements and parameters will be presented in tabular format, including absolute values and changes from baseline (if applicable), by dose cohort and study day.

Adverse events will be coded using the *Medical Dictionary for Regulatory Activities*, and severity of AEs and laboratory abnormalities will be graded using the National Cancer Institute CTCAE, v5.0, with certain exceptions as listed in Table 7. Adverse events will be tabulated by study period, treatment group, system organ class, and preferred term. Incidence tables of participants with AEs will be presented for all AEs by maximum severity, AESIs, SAEs, AEs assessed as related to IMP, and AEs resulting in discontinuation of study dosing.

For the DEP, safety analyses will be performed on the DEP SAS. For the RWP and OLE study parts, safety analyses will be performed on the corresponding SAS, with results being presented for the responder and nonresponder populations as well as overall population.

8.11. Randomization and Blinding

In the RWP, participants will be randomized in a 1:1 ratio to receive either SYNB1934v1 or matching placebo, stratified on screening Phe level (361 to 720 µmol/L, or > 720 µmol/L). Participants, investigators, and the sponsor will be blinded to randomized IMP assignment during the RWP. In the case of an emergency where information regarding treatment assignment would impact the care provided to a participant during the RWP of the study, the investigator will have immediate access to unblind the treatment code in the interactive response system (IXRS). The instructions for unblinding a subject in the IXRS can be found in the IXRS User Guide. In the event unblinding is necessary, the investigator is strongly encouraged to contact the medical monitor to discuss the situation and the subjects medical status prior to unblinding. It is mandatory that any personnel involved in the unblinding or who have access to the unblinded treatment assignment maintain the confidentiality of the information.

During the DEP and RWP, blood Phe levels will be blinded to the participant, investigator and sponsor. During the DEP and RWP, the investigator will receive an email alert from the central laboratory if a blood Phe is > 1.5 × the DEP baseline, if Phe is < 30 µmol/L, or if Tyr is < 20 µmol/L. After the receipt of one of these trigger values, the investigator should arrange for a retest of Phe and Tyr. If the trigger value is confirmed to be out of range, the individual participant will be discontinued from the study.

8.12. Interim Analysis

Once the first [redacted] have attained an iTD and completed the DEP or the [redacted] participant has a baseline DEP and has been in the study [redacted], whichever occurs earlier, the DEP data will be evaluated by an independent DMC. The DMC will use the below pre-specified set of criteria based on both safety and efficacy to determine whether the dose regimen is appropriate or whether any doses should be discontinued as well as whether the study should be stopped for futility. Enrollment will not be paused during the interim analysis.

If a dose level is dropped, participants who are in the DEP and have an iTD corresponding to a dropped dose will not be randomized into the RWP but will instead enter the OLE directly. In addition, participants who are in the OLE at a dose level that has been dropped will be transitioned to the highest dose level retained.

The size of the DEP interim analysis cohort and corresponding criteria for dropping a dose (Table 12) provide reasonable operating characteristics for proper determination if one or more doses should be dropped.

Table 12: DMC Criteria for Dose Changes and Futility

Evaluation by DMC	Criteria to Drop the Dose
1×10^{12} dose	< 20% of the participants who attain an iTD are at the 1×10^{12} dose *OR* > 75% of participants who have 1×10^{12} as their iTD had a greater Phe reduction at their 6×10^{11} dose versus their 1×10^{12} dose.
6×10^{11} dose Note: considered only if the Drop 1×10^{12} Criteria has been met	< 30% of the participants who attain an iTD have 6×10^{11} or 1×10^{12} as their iTD *OR* > 75% of participants who have 6×10^{11} or 1×10^{12} as their iTD had a greater Phe reduction at their 3×10^{11} dose versus their 6×10^{11} dose
Stop for futility	<30% of participants enrolled into the DEP attain an iTD and complete the DEP *OR* < 25% of participants who attain an iTD and complete the DEP are responders

DEP = dose-escalating, open-label period; DMC = data monitoring committee; iTD = individually titrated dose; Phe = phenylalanine

In the event that, based on the interim analysis results utilizing the criteria provided in Table 12, the DMC recommends dropping either the 1×10^{12} or the 1×10^{12} and 6×10^{11} doses, the RWP FAS will be modified as described in Section 8.2.

In addition, the DMC will review interim data at defined intervals in the study. The Sponsor will remain blinded to the subject-level blood Phe levels during the DEP and RWP but will have access to the DEP interim analysis results.

The data from the OLE (Part 3) will be reviewed for safety and tolerability on an ongoing basis.

9. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

Only the sponsor may modify the protocol. Protocol deviations can be made when a participant's safety is compromised. In these circumstances, the investigator should inform the sponsor and the full IRB/IEC/REB within 1 working day after the emergency occurred. All amendments that

have an impact on participant risk or the study objectives or require revision of the ICF must receive approval from the IRB/IEC/REB prior to implementation.

10. DATA RECORDING, RETENTION, AND MONITORING

10.1. Case Report Forms

Data will be collected using source documents through an electronic data capture system at the clinical site. The investigator or designee will record data specified in the protocol using eCRFs. Changes or corrections to eCRFs will be made by the investigator or an authorized member of the study staff according to the eCRF completion guidelines. It is expected that the site will enter all data within 5 business days of a participant visit.

It is the investigator's responsibility to ensure eCRFs are complete and accurate, regardless of whether this responsibility has been delegated in whole or in part. Following review and approval, the investigator or designee will electronically sign and date the pages, which certifies that the investigator has thoroughly reviewed and confirmed all data on the eCRF.

10.2. Data Retention

Data retention practices will follow International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, which note that essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. However, these documents should be retained for a longer period if required by the applicable legal requirements.

10.3. Data Monitoring

This study will be closely monitored by representatives of the sponsor or designee throughout its duration. Monitoring will include personal visits with the investigator and study staff as well as appropriate communications by telephone, teleconference, fax, mail, email, use of the electronic data capture system, or any other method as applicable. It is the monitor's responsibility to inspect eCRFs at regular intervals throughout the study to verify the completeness, accuracy, and consistency of the data and to confirm adherence to the study protocol and Good Clinical Practice (GCP) guidelines. The investigator agrees to cooperate with the monitor to ensure that any problems detected during the course of this study are resolved promptly. The investigator and site will permit study-related monitoring, audits, IRB/IEC/REB review, and regulatory inspection, including direct access to source documents.

It is understood that study monitors and any other personnel authorized by the sponsor may contact and visit the investigator and will be permitted to inspect all study records (including eCRFs and other pertinent data) on request, provided that participant confidentiality is maintained, and that the inspection is conducted in accordance with local regulations.

Every effort will be made to maintain the anonymity and confidentiality of participants during this study. However, because of the experimental nature of the IMP, the investigator agrees to allow representatives of the sponsor and authorized representatives of regulatory authorities to

inspect the facilities used in the conduct of this study and to inspect, for purposes of verification, the hospital or clinic records of all participants enrolled in the study.

10.4. Quality Control and Quality Assurance

Quality control procedures will be conducted according to the sponsor and CRO's internal procedures. The study site may be audited by a quality assurance representative of the sponsor. All necessary data and documents will be made available for inspection.

11. REGULATORY, ETHICAL, AND LEGAL OBLIGATIONS

11.1. Good Clinical Practice

The study will be performed in accordance with the protocol, guidelines for GCP established by the ICH and applicable local regulatory requirements and laws with all clinical study files maintained in accordance with ICH/GCP guidelines.

11.2. Institutional Review Board/Independent Ethics Committee/Research Ethics Board Approval

The investigator must inform and obtain approval from the IRB/IEC/REB for the conduct of the study at named sites, for the protocol, ICF, Investigator Brochure, and any other written information that will be provided to the participants and for any advertisements that will be used. Written approval must be obtained prior to recruitment of participants into the study.

Proposed amendments to the protocol (see Section 9) and aforementioned documents must be submitted to the sponsor for review and approval, then to the IRB/IEC/REB. Amendments may be implemented only after a copy of the approval letter from the IRB/IEC/REB has been transmitted to the sponsor.

In accordance with GCP guidelines, the investigator will be responsible for ensuring that annual updates are provided to the IRB/IEC/REB (or more frequently in accordance with the requirements, policies, and procedures as established by the IRB/IEC/REB) until the study is completed (i.e., finalization of the clinical study report) to facilitate continuing review of the study and that the IRB/IEC/REB is informed about the end of the study. Copies of the update, subsequent approvals, and final letter must be sent to the sponsor.

11.3. Regulatory Authority Approval

The study will be performed in accordance with the requirements of the local health authority (e.g., US FDA, Health Canada) and will also meet all requirements of ICH GCP guidance. Amendments to the protocol will be submitted to the health authority prior to implementation, in accordance with applicable regulations.

11.4. Other Required Approvals

In addition to IRB/IEC/REB and regulatory authority approval, the investigator will be responsible for ensuring that all other required approvals (e.g., approval from the local research

and development board or scientific committee) will be obtained prior to recruitment of participants into the study.

11.5. Informed Consent

Informed consent is a process that is initiated prior to the participants agreeing to participate in the study and continues throughout the participants' study participation. It is the investigator's responsibility (or designee) to obtain written informed consent (the "informed consent form" or ICF) from each participant after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any study procedures are initiated. The investigator or his/her designee will explain the nature of the study to the participant and answer all questions regarding the study. The investigator is responsible for ensuring that the participant fully understands the nature and purpose of the study. Information should be given in both oral and written form whenever possible. No participant should be obliged to participate in the study. The participant must be informed that participation is voluntary, that they may withdraw at any time, and that choosing not to participate will not affect the care the participant will receive.

Each participant should be given a copy of the ICF and associated materials. The original copy of the signed and dated ICF must be retained at the site and is subject to inspection by representatives of the sponsor, regulatory authorities, or IRB/IEC/REB. If any amendments occur throughout the course of the study that affect the ICF (i.e., when new study procedures or assessments have been added), all active participants should be reconsented using the same process for the initial consent.

11.6. Participant Confidentiality

The investigator must ensure that the participant's privacy is maintained and that all necessary and appropriate measures consistent with applicable law will be employed to safeguard their personal, health, and medical information. On eCRFs and other documents submitted to the sponsor, participants will be identified by their assigned participant number. Documents that are not submitted to the sponsor (e.g., signed ICFs) should be kept in a confidential file by the lead investigator.

All personal data gathered in this trial will be treated in the strictest confidence by investigators, monitors, and all associated personnel. No data will be disclosed to any third party without the express permission of the participant concerned, except that the investigator shall permit authorized representatives of the sponsor, regulatory authorities, and the IRB/IEC/REB to review the portion of the participant's medical record that is directly related to the study. As part of the required content of the ICF, the participant must be informed that his/her records will be reviewed in this manner.

For trials being conducted under Regulation EU No 536/2014, as per Annex I, D (17am), the contract between the Sponsor and vendors or trial sites will specify the responsibilities of the parties related to data protection, including the handling of data security breaches and respective communication and cooperation of the parties.

11.7. Study Confidentiality and Disclosure of Information

Information concerning the study, the progress of the study, protocol, processes, assessments, IMP, scientific data, or other non-public information related to the study or the sponsor and its representatives is confidential and remains the property of the sponsor. The lead investigator and his or her designees may use this information for the purposes of the study only.

It is understood by the lead investigator that the sponsor will use information obtained in this clinical study in connection with the clinical development program, and therefore may disclose it as it sees fit, including to other clinical investigators and to regulatory authorities. In order to allow the use of the information derived from this clinical study, the lead investigator understands that he/she has an obligation to provide complete test results and all data obtained during this study to the sponsor.

Verbal or written discussion of the progress of the study, or the study results, prior to study completion and full reporting, should only be undertaken with written consent from the sponsor.

11.8. Publication of Study Data

The sponsor encourages the scientific publication of data from clinical research studies in a relevant peer-reviewed journal. However, investigators may not present or publish partial or complete study results individually without participation of the sponsor. The lead investigator and sponsor may propose appropriate scientific manuscripts or abstracts from the study data. All proposed publications must be reviewed and commented on by the sponsor before submission for publication. The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the sponsor in connection with this study. These procedures are in place to ensure (a) coordination of study data publication, (b) adequate review of data for publication against the validated study database for accuracy, and (c) that confidentiality of sponsor business information or participant personal information is maintained.

Qualification of authorship will follow the requirements of the International Committee of Medical Editors (www.icmje.org). The names of investigators and sponsor representatives responsible for designing the study and analyzing the results will be included in the publication(s). This custom can be adjusted upon mutual agreement of the authors and Synlogic.

In addition, this clinical trial must be registered with ClinicalTrials.gov, which will be done by the sponsor or its representative and will be registered in any applicable international database or website as required by law or regulation. The results of and data from this study belong to Synlogic.

11.9. Ethical Standards

We are committed to designing, implementing, conducting, analyzing, and reporting these trials in compliance with the highest ethical and scientific standards. Protection of participant safety is the overriding concern in the design of clinical trials. In all cases, Synlogic clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the consensus ethical principles that have their origin in the Declaration of Helsinki.

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APPENDIX 1. CLINICAL LABORATORY TESTS

Hematology (CBC with differential)	Basophils% Basophils Eosinophils% Eosinophils Hematocrit Hemoglobin Lymphocytes% Lymphocytes Mean corpuscular hemoglobin Mean corpuscular volume Monocytes% Monocytes Neutrophils% Neutrophils Platelet count Red blood cells White blood cells	**Serum chemistry**	Glucose BUN Creatinine with eGFR Sodium Potassium Chloride Calcium Total protein Albumin Fractionated bilirubin (total direct and indirect) Follicle-stimulating hormone (for postmenopausal women only)[a] Alkaline phosphatase Aspartate aminotransferase Alanine aminotransferase Pregnancy (for WOBCP only) Glucose C-reactive protein
Urinalysis	Specific gravity pH Glucose Bilirubin Ketones Occult blood Protein Nitrite Leukocyte esterase Pregnancy (for WOBCP only)		

Abbreviations: BUN = blood urea nitrogen; eGFR = estimated glomerular filtration rate; WOBCP = women of childbearing potential.

a Performed at screening only.

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Approval Task Task Verdict: Approved	[redacted]

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