**CLINICAL STUDY SYNB1020-CP-002**

**Protocol Version 6: 27 November 2018**

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| **Study Title:** | A Randomized, Double-blind, Placebo-controlled Study to Assess the Safety, Tolerability, and Pharmacodynamics of SYNB1020 in Hepatic Insufficiency and Cirrhosis Patients |
| **Study Number:** | SYNB1020-CP-002 |
| **Study Phase:** | Phase 1b/2a |
| **Product Name:** | SYNB1020 |
| **Indication:** | Cirrhosis Patients |
| **Sponsor:** | Synlogic, Inc. |

**Synlogic, Inc.**

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# SIGNATURE PAGE (SPONSOR)

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1020-CP-002 Version 6, dated 27 November 2018, and agree to meet all obligations of Synlogic, Inc. as detailed in all applicable regulations and guidelines. In addition, I will ensure that the Principal Investigators are informed of all relevant information that becomes available during the conduct of this study.

_____ _____

[redacted]

# PRINCIPAL INVESTIGATOR AGREEMENT

I have read and understand the contents of the clinical protocol for Clinical Study SYNB1020-CP-002 Version 6, dated 27 November 2018, 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 patients in accordance with the following:

- International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use 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 9 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.

My signature also acknowledges that:

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

Name of Principal Investigator

Signature of Principal Investigator

Date

# PROTOCOL SYNOPSIS

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| **Name of Sponsor/Company:**<br>Synlogic, Inc. | |
| **Name of Investigational Product:**<br>SYNB1020 | |
| **Name of Active Ingredient:**<br>SYNB1020 | |
| **Title of Study:**<br>A Randomized, Double-blind, Placebo-controlled Study to Assess the Safety,Tolerability, and Pharmacodynamics of SYNB1020 in Hepatic Insufficiency and Cirrhosis Patients | |
| **Study Center:** Up to 10 centers in the United States | |
| **Objectives and Endpoints** | The primary objective is to evaluate the safety and tolerability of SYNB1020 following multiple doses in hepatic insufficiency and cirrhosis patients. The endpoints related to this objective include the nature and frequency of adverse events (AEs), measurement of vital signs and laboratory assessments (e.g., blood chemistry, complete blood count [CBC], liver function tests, international normalized ratio [INR], and urinalysis), and electrocardiograms (ECGs).<br>Secondary:<br>• SYNB1020 kinetics in feces (measured with qualitative and quantitative polymerase chain reaction [qPCR] fecal assays) following dosing<br>• Change from baseline of venous ammonia, including fasting and 24-hour area under the curve (AUC), following SYNB1020 dosing in subjects with hyperammonemia<br>[redacted] |
| **Design** | This Phase 1b/2a, randomized, double-blinded placebo-controlled study will evaluate the safety, tolerability, and pharmacodynamics of SYNB1020 in hepatic insufficiency and cirrhosis patients, with dosing of the investigational product (IP) |

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| | administered in an inpatient unit and subsequent outpatient follow-up for SYNB1020 clearance. This study consists of two parts:<br>Part 1:<br>A sentinel open-label cohort of subjects with cirrhosis and Model for End-Stage Liver Disease (MELD) score <12 will be admitted to an inpatient facility for a run-in diet, baseline assessments, IP administration, safety monitoring, and collection of blood, urine, and fecal samples for evaluation of safety, tolerability, and pharmacokinetics and PD evaluations. Once the safety and tolerability have been established in Part 1, enrollment will be opened to subjects in Part 2.<br>Part 2:<br>Part 2 of the trial will comprise a randomized, double-blinded, placebo-controlled study in subjects with cirrhosis and hyperammonemia. Subjects may be pre-screened for eligibility based on medical history and a single fasting spot venous ammonia measurement. Eligible subjects with elevated fasting spot venous ammonia will then undergo full screening within 7 days of pre-screening. Eligible subjects will be admitted to an inpatient facility for a run-in diet and 24-hour ammonia profile, and those with an elevated 24-hour ammonia AUC (>1.2 × the upper limit of normal [ULN]) will proceed with randomization, IP administration, safety monitoring, and collection of blood, urine, and fecal samples for pharmacokinetic and pharmacodynamic (PD) evaluations. |
| **Study Regimens** | Subjects will receive a calorie and protein controlled diet during inpatient monitoring, with specified macronutrient content based on individual subject food preferences. Subjects will be expected to consume the entirety of their meals and not consume any external foods for the duration of their inpatient study participation. Diet consumption will be recorded.<br>In addition, subjects will be administered a histamine-2 receptor (H2) inhibitor to reduce stomach acid, comprising oral ranitidine 150 mg administered twice daily (BID). Subjects already on a proton pump inhibitor (PPI) will not be switched to ranitidine but will continue their prescribed PPI regimen for the duration of the study.<br>Part 1 is open-label; in Part 2 both subjects and investigators will be blinded to randomized IP assignment.<br>**Inpatient Monitoring**<br>Part 1:<br>Each subject will be admitted to the inpatient unit after an overnight fast on the morning of Day -5. Beginning on Day -5, subjects will initiate a controlled diet and ranitidine. Days -5 through -1 will be a run-in phase to allow for dietary stabilization on the controlled diet. On Day -2, all subjects will undergo baseline 24-hour ammonia profile with 8 measurements (AUC), baseline clinical laboratory sampling and other safety measurements, FibroScan® measurement, and baseline hepatic encephalopathy grading [redacted]. Subjects will receive up to $5 \times 10^{11}$ colony-forming units (CFU) of SYNB1020 administered 3 times per day (TID) immediately after meals from Days 1 through 6 (including mid-day and evening doses on Day 6). Fasting ammonia levels will be measured daily. On Day 5, subjects will undergo ammonia AUC assessment. On Day 6, subjects will undergo ammonia AUC assessment, repeat hepatic encephalopathy grading [redacted], as well as 24-hour urine and blood collection. Subjects will be monitored in the inpatient setting for at least 12 hours |

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| | following completion of dosing, with discharge on Day 7 for clinically stable subjects. Stool samples will be collected daily during the inpatient period.<br>Part 2:<br>Each subject will be admitted to the inpatient unit after an overnight fast on the morning of Day -5. Beginning on Day -5, subjects will initiate a controlled diet and ranitidine. Days -5 through -1 will be a run-in phase to allow for dietary stabilization on the controlled diet. On Day -2, all subjects will undergo baseline 24-hour ammonia profile with 8 measurements (AUC), baseline clinical laboratory sampling and other safety measurements, FibroScan® measurement, and baseline hepatic encephalopathy grading [redacted]. Subjects whose 24-hour AUC venous ammonia measurement is above 1.2 × ULN will undergo computer-generated randomization on Day -1 in a 1:1 ratio to receive either up to $5 \times 10^{11}$ CFU of SYNB1020 or matching placebo administered TID immediately after meals for 6 days. Subjects will receive IP dosing from Days 1 through 6 (including mid-day and evening doses on Day 6). Fasting ammonia levels will be measured daily. On Day 5, subjects will undergo ammonia AUC assessment. On Day 6, subjects will undergo ammonia AUC assessment, repeat hepatic encephalopathy grading [redacted], as well as 24-hour urine and blood collection. Subjects will be monitored in the inpatient setting for at least 12 hours following completion of dosing, with discharge on Day 7 for clinically stable subjects. Stool samples will be collected daily during the inpatient period.<br>**Outpatient Follow-up**<br>One week (7 ± 1 days) after discharge from the inpatient unit, subjects will return for clinical evaluation and bring a 1-week post-dose fecal sample. Subjects will be provided diary cards for collection of AE data. Feces will continue to be collected weekly for the first 6 weeks following discharge and biweekly until a subject has a negative SYNB1020 fecal test for up to 10 weeks following completion of study dosing. Subjects who remain colonized with SYNB1020 after 10 weeks following the last dose will be treated with a 3-day course of oral ciprofloxacin (or other antibiotic for subjects allergic to ciprofloxacin). A SYNB1020 fecal test will be performed 3 to 5 days following completion of antibiotic treatment solely for research purposes and will not guide subject follow-up decision making. An AE assessment will be performed by telephone at least 7 days after completion of antibiotic treatment. If no AEs are reported, subjects will not require any further follow-up and will be considered to have completed the study requirements. If AEs are reported, subjects will be advised to return to the inpatient facility for a comprehensive physical examination, laboratory assessment, and AE causality determination. |
| **Study Population** | This study will comprise adult male and female patients with hepatic insufficiency and cirrhosis. |
| **Duration of Study Participation** | The maximum time of study participation for a subject is 133 days, including pre-screening (up to 7 days), screening (up to 45 days), inpatient monitoring (12 days), and outpatient follow-up (up to 70 days after last dose). |
| **Analytical and Statistical Methods** | **Sample Size Determination**<br>Part 1: The sample size for the sentinel cohort (6 subjects) is primarily designed for empirical evaluation of safety and tolerability in subjects with cirrhosis.<br>Part 2: A sample of 24 subjects must complete all 3 ammonia AUC intervals |

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| | (baseline, Day 5, and Day 6) to detect a 20% reduction in average daily ammonia ($AUC_{0-24}$/24, from a baseline of 70 µmol/L) with an approximate significance level of 10% and an approximate power of 90%. Significance and power are approximate based on the assumptions below. The number of subjects assumes that the standard deviation (SD) of spot ammonia is 36.4 µmol/L, half of the variance is between subject, and that the SD for average daily ammonia is as little as half that of spot ammonia. The spot ammonia SD is based on a weighted average of SD observed in a prior study of ammonia lowering in a similar population (Ghabril 2013). The variability of AUC may be 50% lower than spot ammonia based on the Food and Drug Administration (FDA) summary basis of approval for glycerol phenylbutyrate for urea cycle disorders (Center for Drug Evaluation and Research 2012).<br><br>Due to uncertainty in the assumptions of decrease in variability in AUC of ammonia relative to spot measurements in this population, up to 2 interim analyses for sample size re-estimation may occur after at least 12 subjects have completed all ammonia AUC assessments, and the total number of subjects in Part 2 of the study may be increased up to 40 at either point based on the SD of data observed. Additional subjects may be enrolled to ensure that at least the planned number of evaluable subjects complete the study.<br><br>**Safety Analysis**<br><br>Safety will be evaluated by continuous monitoring of AEs, vital signs, clinical laboratory measurements (including MELD score components), ECGs, and physical examinations. After completion of Part 1, a Safety Review Committee will conduct a safety evaluation (including any available data on AEs, laboratory measurements, vital signs, and ECGs up to 7 days after the last dose of SYNB1020) before opening Part 2 for enrollment. In Part 2, the Safety Review Committee will conduct blinded safety evaluations at predetermined study time points and ad hoc, as needed, and will be defined in the Safety Review Committee charter.<br><br>Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®), and severity of AEs and laboratory abnormalities will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. Adverse events will be tabulated by system organ class (SOC) and preferred term. Incidence tables of subjects with AEs will be presented for all AEs by maximum severity, serious adverse events (SAEs), AEs assessed as related to SYNB1020/placebo, and AEs resulting in discontinuation of study dosing.<br><br>**Microbiotic-Kinetic Analysis**<br><br>Feces will be collected daily following dosing through Day 7 for analysis of SYNB1020 transit through the gastrointestinal tract. In Part 1, the 24-hour fecal sample will be weighed, homogenized, and a sample analyzed for SYNB1020 by qPCR. In Part 2, a fecal sample from the first void of the day will be collected using a collection kit. The following endpoints will be calculated from the serial fecal sampling from each subject, if data support calculation:<br><br>• $T_{lag}$ – the time of the first detectable fecal SYNB1020<br>• $T_{ss}$ – the time to steady-state<br>• $q_{max}$ – the maximum observed fecal SYNB1020 qPCR signal |

- $T_{last}$ – the time of the last detectable fecal SYNB1020
- Half-life – the time for the fecal SYNB1020 qPCR signal to decline by 50%

A sample will be considered negative for the purposes of SYNB1020 clearance if it is below the limit of quantification or estimated to be below 0.1% of the administered dose.

The microbiotic-kinetic parameters of SYNB1020 will be summarized descriptively.

**Pharmacodynamic Analysis**

Urine and blood samples will be collected at screening and on study both before and after administration of SYNB1020 or placebo. The following laboratory measurements will be performed to evaluate the preliminary PD of SYNB1020:

- Venous ammonia (morning fasting and 24-hour profile to calculate AUC)

[redacted]

- Bristol stool form scale (BSFS) for each bowel movement

[redacted]

The PD parameters of SYNB1020 will be summarized descriptively.

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# LIST OF ABBREVIATIONS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| AE | adverse event |
| ALT | alanine aminotransferase |
| aPTT | activated partial thromboplastin time |
| Arg | arginine |
| ArgA | N-acetylglutamate synthase |
| *argA* | N-acetylglutamate synthase gene |
| *argA*$^{fbr}$ | feedback-resistant N-acetylglutamate synthase |
| ArgB | acetylglutamate kinase |
| ArgC | N-acetylglutamylphosphate reductase |
| ArgD | N-acetylornithine aminotransferase |
| ArgE | acetylornithine deacetylase |
| ArgFI | ornithine carbamoyltransferase |
| ArgG | arginosuccinate synthase |
| ArgH | arginosuccinate lyase |
| ArgR | transcriptional repressor |
| *argR* | arginine repressor gene |
| *ΔargR* | arginine repressor gene deletion |
| AST | aspartate aminotransferase |
| Asp | aspartic acid |
| AUC | area under the curve |
| $AUC_{0\text{-}24}$ | area under the curve from time 0 to 24 hours |
| BID | bis in die (twice daily) |
| BSFS | Bristol stool form scale |
| CarAB | carbamoylphosphate synthetase |
| CBC | complete blood count |
| CFU | colony-forming unit(s) |
| CRO | Contract Research Organization |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CV | curriculum vitae |
| DRI | dietary reference intakes |
| ECG | electrocardiogram |
| EcN | *Escherichia coli* Nissle 1917 |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| *E. coli* | *Escherichia Coli* |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| eGFR | estimated glomerular filtration rate |
| EOS | End of Study |
| FDA | Food and Drug Administration |
| FNR | fumarate and nitrate reductase |
| FSH | follicle-stimulating hormone |
| g | gram(s) |
| GCP | Good Clinical Practice |
| GI | gastrointestinal |
| Gln | Glutamine |
| GLP | Good Laboratory Practice |
| Glu | Glutamate |
| h | hour(s) |
| H2 | histamine-2 receptor |
| HE | hepatic encephalopathy |
| $HCO_3$ | Bicarbonate |
| HIV | human immunodeficiency virus |
| HRS | hepato-renal syndrome |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| [redacted] | [redacted] |
| INR | international normalized ratio |
| IP | investigational product |
| IRB | Institutional Review Board |
| KG | Ketoglutarate |
| kg | Kilogram |
| LFT | liver function test |
| $m^2$ | meter(s) squared |
| malEK | malE = maltose gene, malK = maltose transpose complex, ATP-binding subunit gene |
| MedDRA® | Medical Dictionary for Regulatory Activities |
| MELD | Model for End-Stage Liver Disease |
| mg | milligram(s) |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| µg | microgram(s) |
| min | minute(s) |
| MK | microbiotic-kinetic |
| µL | microliter(s) |
| mL | milliliter(s) |
| mM | millimolar(s) |
| µmol | micromole(s) |
| MOPS | 3-(N-morpholino) propanesulfonic acid |
| mRNA | messenger ribonucleic acid |
| ms | millisecond(s) |
| MTD | maximum tolerated dose |
| NCI | National Cancer Institute |
| $NH_3$ | ammonia |
| NHP | nonhuman primate |
| No. | number |
| $O_2$ | oxygen |
| OTC | ornithine transcarbamylase |
| PD | pharmacodynamics(s) |
| $P_{fnrS}$ | fumarate and nitrate reductase gene S promoter |
| PO | oral administration |
| PPI | proton pump inhibitor |
| PT | prothrombin time |
| QD | quaque die (once daily) |
| $q_{max}$ | maximum observed fecal SYNB1020 qPCR signal |
| (q)PCR | (quantitative) polymerase chain reaction |
| QTc | QT interval corrected for heart rate |
| QTcF | QT interval corrected for heart rate using Fridericia's formula |
| SAE | serious adverse event |
| SAD | single ascending dose |
| SAF | safety population |
| SAP | statistical analysis plan |
| SBP | spontaneous bacterial peritonitis |
| SD | standard deviation |
| SOC | system organ class |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| SUSAR | serious, unexpected, suspected adverse reaction |
| TAA | thioacetamide |
| TEAE | treatment-emergent adverse event |
| *thyA* | thymidylate synthase |
| *ΔthyA* | thymidylate synthase deletion |
| TID | ter in die (3 times daily) |
| $T_{lag}$ | time of the first detectable fecal SYNB1020 |
| $T_{last}$ | time of the last detectable fecal SYNB1020 |
| [redacted]α | [redacted] |
| $T_{ss}$ | time to steady-state |
| ULN | upper limit of normal |
| US(A) | United States (of America) |

# 1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The Principal Investigator is the person responsible for the conduct of the study at the investigational site. A sub investigator is any member of the clinical study team designated and supervised by the Principal Investigator to perform critical study-related procedures and/or to make important study-related decisions.

Prior to study initiation, the Principal Investigator at each site must provide to Synlogic, Inc. (also referred to as "Synlogic" or "Sponsor") a signed protocol signature page, a fully executed and signed United States (US) Food and Drug Administration (FDA) Form 1572, a current curriculum vitae (CV) and medical license and a financial disclosure form. Financial disclosure forms, current CVs and medical licenses must also be provided for all sub investigators listed on Form 1572 who will be directly involved in the evaluation of subjects.

The study will be administered and monitored by employees or representatives of Synlogic in accordance with all applicable regulations. Clinical research associates will monitor each site on a periodic basis and perform verification of source documentation for each subject. Synlogic or designee will be responsible for ensuring timely reporting of expedited serious adverse event (SAE) reports to regulatory authorities and Investigators.

# 2. INTRODUCTION

## 2.1. Background of Hepatic Encephalopathy

The proposed target indication for the clinical development of SYNB1020 is hepatic encephalopathy (HE), a potentially life-threatening condition.

Chronic liver diseases affect approximately 150,000 new patients in the United States (US) each year, nearly 20% of whom present with cirrhosis.[1] The primary function of the liver is to convert toxins, such as ammonia, into nontoxic molecules, such as urea and glutamine to enable safe elimination from the body. Thus, patients with impaired liver function are susceptible to an excess accumulation of toxins in the blood stream, which presents as HE in approximately 55% of patients with chronic liver disease.[2] Hepatic encephalopathy comprises a heterogenous group of disorders characterized by declining neurocognitive function, and while many HE symptoms may be reversible with appropriate treatment, persistent impairment of cognitive function may occur.[1,3]

The classification of HE severity is based largely on a patient's mental state at presentation and is often graded according to the West Haven criteria, which are based on clinical findings in changes of consciousness, intellectual function, and behavior (see Appendix 2). Covert HE, also known as minimal HE, is difficult to diagnose and is often observed in patients with cirrhosis who show no obvious neurologic symptoms and signs. However, more sensitive psychomotor testing reveals subtle changes in attention, psychomotor speed, and executive decision-making, which have a profound impact on daily life. Such changes result in difficulty concentrating, forgetfulness, changes in personality or behavior, and poor sleep.[3,4] The more severe form of HE, referred to as overt HE, is associated with obvious mental disorientation and physical symptoms (e.g., lethargy, seizures, tremors, organ failure, or brain swelling) that arise suddenly over several days or hours and may induce a coma or even death.[3,4] Overt HE is associated with a poor prognosis, with 1-year survival estimates ranging from 20% to 54%.[5] Due to difficulty in diagnosis, the prognosis of covert HE is less understood but has been associated with poor quality of life, reduced work performance, increased risk of road traffic accidents, and a higher risk of developing potentially severe overt HE episodes.[4,6,7]

The pathogenesis of HE is believed to be largely attributable to hyperammonia, i.e., the inadequate de-toxicification and accumulation of ammonia, as well as the inflammatory process.[8] The metabolism and subsequent renal elimination of ammonia, a highly toxic substance produced in the body as a by-product of protein metabolism, is predominantly reliant on a functional liver. While ammonia may also be minimally metabolized in the muscle and by the brain in patients with impaired liver function, the excessive ammonia levels (i.e., hyperammonemia) often overwhelm the metabolic capacity of brain tissue and result in an increased risk of brain edema, herniation, and death for patients with HE.[3,9]

### 2.1.1. Unmet Medical Need in Hepatic Encephalopathy

The current standard of care for HE includes reducing blood ammonia levels using lactulose, a nonabsorbable disaccharide, administered concurrently with antibiotics to reduce inflammation.[4,5,8] In addition, rifaximin, a broad spectrum antibiotic, is approved as a treatment

to reduce the risk of overt HE recurrence in adults.[10] Rifaximin has demonstrated improved maintenance of remission, reduced hospitalizations over 6 months, and improved quality of life in patients with HE.[11] Dietary modification and supplementation may also be implemented for patients with HE. While restrictions in dietary protein intake were historically recommended to reduce the amount of ammonia required to be metabolized, current recommendations include adequate (but not excessive) consumption of protein and other nutrients to avoid muscle wasting, as muscles are able to metabolize ammonia and may alleviate the burden of ammonia metabolism required by the brain.[4] Probiotics may also be administered as a component of HE therapy, as they have demonstrated some benefit in the treatment of HE through modulation of bacterial growth in the gut.[4,8]

When these management approaches fail to control HE, patients with liver disease may be candidates for a potentially curative liver transplantation. However, the benefits of transplantation must be carefully examined to ensure that they outweigh the added exposure to potentially life-threatening risks, such as suppression of the immune system and its consequences, including fatal viruses (e.g., Epstein-Barr, cytomegalovirus) and lymphoproliferative diseases. Furthermore, many patients succumb to their underlying disease while waiting for an eligible liver for transplantation.[12]

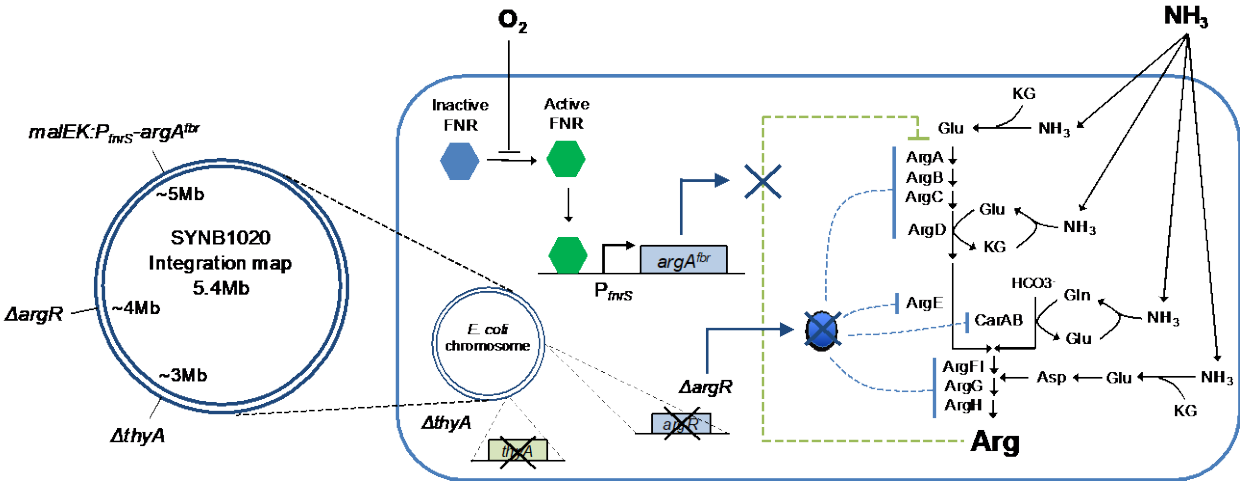
Morbidity and mortality associated with HE remain high, and hospitalizations for HE impose a high burden on community resources.[1,8] While overt HE is associated with substantially more severe symptoms, adequate management of covert disease is also important in order to maintain optimal neurocognitive function and stave off the more severe manifestations of the disease. Although rifaximin and lactulose are approved for overt HE, no treatments are currently approved for covert disease.

## 2.2. Investigational Medicinal Product

SYNB1020 is an orally administered live biotherapeutic product that is a genetically modified strain of *Escherichia coli* Nissle 1917 (EcN) designed to consume ammonia and produce arginine in the intestinal tract (Figure 1). Two modifications are made to the EcN chromosome within the arginine biosynthetic pathway: deletion of the arginine repressor gene (*argR*), and insertion of a gene that encodes a feedback resistant version of N-acetylglutamate synthase ($argA^{fbr}$) under the control of an inducible promoter. Removing the *argR* gene prevents expression of the transcriptional repressor ArgR. ArgR inhibits the expression of all genes in the arginine biosynthetic pathway when arginine is abundant; therefore, a loss of ArgR results in the expression of all of the arginine biosynthetic enzymes. In unmodified EcN, the N-acetylglutamate synthase (ArgA) enzyme can be inhibited by arginine in order to prevent excess arginine production. In contrast, the $argA^{fbr}$ enzyme cannot be inhibited by arginine; therefore this enzyme increases arginine production in cells lacking ArgR. In SYNB1020, the promoter fumarate and nitrate reductase gene S ($P_{fnrS}$) controls the expression of the $argA^{fbr}$ gene. The $P_{fnrS}$ promoter inhibits expression of the $argA^{fbr}$ gene in aerobic environments, and only allows expression of the gene when the cell experiences micro-aerobic or anaerobic environments. Thus, excess arginine is only produced in environments where oxygen is limiting, such as the mammalian gut. In order to limit unwanted growth of the strain, the thymidylate synthase (*thyA*) gene is deleted. Because of this deletion, SYNB1020 can only grow in thymidine-rich environments.

SYNB1020 and its parent strain, EcN, were evaluated for antibiotic susceptibility using established methodology and interpretive criteria against a panel of 16 common antibiotics (see the Investigator's Brochure). Both strains were susceptible to all antibiotics tested, which is consistent with analyses from whole genome sequencing that failed to identify any antibiotic resistance elements in these strains.

**Figure 1: Schematic of Key SYNB1020 Components**




Abbreviations: Arg = arginine; ArgA = N-acetylglutamate synthase; *argA* = N-acetylglutamate synthase gene; *argA*$^{fbr}$ = feedback resistant N-acetylglutamate synthase gene; ArgB = acetylglutamate kinase; ArgC = N-acetylglutamylphosphate reductase; ArgD = N-acetylornithine aminotransferase; ArgE = acetylornithine deacetylase; ArgFI = ornithine carbamoyltransferase; ArgG = arginosuccinate synthase; ArgH = arginosuccinate lyase; *ΔargR* = arginine repressor gene deletion; Asp = aspartic acid ; CarAB = carbamoylphosphate synthetase; *E. coli* = *Escherichia coli*; FNR = fumarate and nitrate reductase; Gln = glutamine; Glu = glutamate;
$HCO_3$ = bicarbonate; KG = ketoglutarate; malEK = malE = maltose gene, malK = maltose transpose complex, ATP-binding subunit gene; $NH_3$ = ammonia; $O_2$ = oxygen; $P_{fnrS}$ = fumarate and nitrate reductase regulator sensor promoter;
*ΔthyA* = thymidylate synthase deletion

The rationale for development of SYNB1020 was to create a commensal strain of EcN that would produce excess arginine and continuously absorb excess ammonia where it is naturally produced, in the colon, before it can be absorbed into the blood. Because ammonia is produced in the gastrointestinal (GI) tract, a gut commensal bacterial strain programmed to consume ammonia may be an ideal therapeutic to reduce the levels of excess ammonia in the blood of patients with liver disease and therefore reduce hyperammonemia in patients with HE.[13-16] The endogenous arginine pathway was chosen for augmentation because 4 molecules of ammonia are consumed for every molecule of arginine produced, which may result in a more effective channel for ammonia assimilation than other amino acid biosynthetic pathways.[17]

### 2.2.1. Nonclinical Studies

#### 2.2.1.1. Nonclinical Pharmacology

The SYNB1020 strain was evaluated with relevant comparators in a series of in vitro assays of arginine biosynthesis as well as in vivo studies designed to measure ammonia consumption. Nonclinical evaluation included an antibiotic-resistant strain, SYNB1010, that was designed with the same gene modifications as SYNB1020 except for the nucleotide changes corresponding to

the kanamycin resistance gene. For a comprehensive description of the nonclinical development program, refer to the SYNB1020 Investigator's Brochure.

#### 2.2.1.2. In Vitro Characterization of SYNB1010/SYNB1020

As a biocontainment measure, SYNB1020 and precursor strains were engineered with a deletion of the *thyA* gene encoding thymidylate synthase to create a thymidine auxotroph. At least 2 mM of thymidine was found to be required for continuous growth of these strains, and at lower concentrations the strains grew for a short period of time and then began to die. Concentrations of thymidine in intestinal fluid, urine, and blood are very low (< 0.03 mM). Therefore, SYNB1020 is not expected to grow in intestinal fluids, blood, or urine.

##### 2.2.1.2.1. In Vivo Characterization of SYNB1010/SYNB1020

The distribution of SYNB1010 following oral administration was studied in normal mice. It was found that within 1 hour of oral dosing of mice with $10^{10}$ colony-forming units (CFU), peak levels of bacteria were found in the distal small intestine and cecum. The cecum and colon remained the sites of highest concentration of bacterial load over the 30 hours post-dosing. Oral dose levels of $10^7$ to $10^{10}$ CFU of SYNB1010 on Days 1 and 2 were evaluated for recovery in mouse feces from Days 1 to 5. Higher doses were associated with higher levels of recovery, with the greatest amounts recovered on Days 2 and 3 after dosing. Complete clearance of detectable SYNB1010 requires longer than 3 days following the last oral dose for dose levels greater than $10^7$ CFUs of bacteria.

Following oral dosing of mice with $10^{10}$ CFU of SYNB1010, SYNB1010 recovered from cecum and colonic contents was metabolically active and converted ammonia to arginine in ex vivo studies at levels similar to those measured in vitro for the strain.

##### 2.2.1.2.2. Pharmacodynamic Studies in Thioacetamide Induced Liver Injury Mouse Model

In vivo efficacy of SYNB1020 was studied in thioacetamide (TAA)-treated BALB/c mice (N=10/group). Following 4 weeks of TAA administration (150 mg/kg 3 times/week) mice were switched to 70% high protein diet for 3 days in order to induce hyperammonemia. SYNB1020 was orally administrated at increasing doses. Administration of $1 \times 10^9$ to $1 \times 10^{10}$ CFU of SYNB1020 lowered blood ammonia levels in a dose dependent manner, with statistically significant ammonia reduction at $1 \times 10^{10}$ CFU of SYNB1020 (from 144 ± 10.9 µg/dL to 84 ± 11.1 µg/dL).

##### 2.2.1.2.3. Pharmacodynamic Studies in *spf$^{ash}$* Mouse Model with Orthinine Transcarbamylase Deficiency

In vivo efficacy of the bacterial strains was studied in the *spf$^{ash}$* mouse model of ornithine transcarbamylase (OTC) deficiency, a subset of hyperammonemia that results from a defect in the activity of one of the enzymes of the urea cycle.[18] Mice exhibit a deficiency in OTC messenger ribonucleic acid (mRNA), and affected mice exhibit increased blood levels of ammonia observed upon feeding with an extremely protein-rich diet. Mice dosed with SYNB1010 exhibited significantly decreased blood ammonia levels. Hyperammonemic mice dosed with SYNB1010 showed an increased survival benefit.

#### 2.2.1.3. Nonclinical Toxicology

##### 2.2.1.3.1. Nonhuman Primate Study

A non-Good Laboratory Practice (non-GLP) repeat-dose safety and tolerability preclinical safety study in the nonhuman primate (NHP) was conducted. Female cynomolgus monkeys, 2 to 8 years of age, were dosed once daily (QD) for 28 days with SYNB1010 at a maximum dose of $10^{12}$ CFU/animal followed by a bicarbonate flush. Control animals received bicarbonate solution only. SYNB1010 was well tolerated; no study-compound-related mortality occurred and no test article-related effects were identified on clinical observations, body weight, or clinical pathology assessments including hematology, coagulation, and clinical chemistry. In the SYNB1010-dosed animals, no bacteria were detected in the feces after Day 7 post-dosing.

##### 2.2.1.3.2. 28-day Good Laboratory Practice Mouse Toxicology Study

To evaluate the potential toxicity of SYNB1020, a 28-day, GLP toxicology study was conducted in male and female CD-1 mice. The study evaluated twice daily (BID) oral gavage (PO) dose administration of SYNB1020 for 4 weeks followed by a 2-week recovery period. The maximum dose was $1.56 \times 10^{11}$ CFU/day, which is the highest feasible dose in this species and corresponds to a human dose of $2.3 \times 10^{14}$ based on the human and mouse GI volume ratio of 1100.[19] There was no test-article-related mortality, no clinical observations, and no pathological findings on necropsy. Low levels of SYNB1020 DNA were detectable in stomach ileum and colon 24 hours following the last dose but were not detected outside the GI tract (spleen, liver, bladder, gonads) in animals correctly dosed by oral gavage.

#### 2.2.1.4. Summary of the Nonclinical Development Program

In summary, SYNB1020/1010 have been evaluated in a preclinical efficacy and safety program that includes in vitro characterization that demonstrates increased ammonia consumption and arginine production as well as the growth-limiting effect of thymidine auxotrophy. SYNB1020 does not contain any antibiotic resistance genetic markers and has demonstrated susceptibility to a wide range of antibiotics. In vivo, kinetics in cynomolgus monkeys demonstrated that the strain does not colonize and is cleared from the feces within a week following discontinuation of dosing. In the nonclinical safety program, SYNB1020 was evaluated in a GLP toxicology study in mice and a closely related strain, SYNB1010, was evaluated in a non-GLP toxicology study in NHPs. There were no toxicological findings after 28 days of dosing at dose levels of up to $10^{11}$ CFU in mice and $10^{12}$ CFU in the NHP.

### 2.2.2. Clinical Studies

Study SYNB1020-CP-002 represents the second clinical investigation of SYNB1020. One study of SYNB1020 is ongoing in healthy adult male and female volunteers (see Section 2.2.2.1). In addition, a large body of evidence is available for the clinical safety and efficacy of *E. coli* Nissle, the parent strain of SYNB1020 (see the SYNB1020 Investigator's Brochure).

#### 2.2.2.1. Study SYNB1020-CP-001

Study SYNB1020-CP-001 is an ongoing Phase 1, dose-escalating, randomized, double-blinded study designed to determine the maximum tolerated dose (MTD) of single and multiple doses of

SYNB1020 in placebo-controlled cohorts of male and female healthy adult subjects. As of 30 October 2017, 52 subjects (40 male, 12 female) aged between 21 and 64 years were enrolled in Study SYNB1020-CP-001 and received at least 1 dose of SYNB1020 or matching placebo (Table 1).

**Table 1: Study Enrollment in Study SYNB1020-CP-001**

| Regimen | SYNB1020 Dose | No. Subjects Enrolled (SYNB1020/Placebo) |
|---|---|---|
| Single Day | Single dose of $2 \times 10^9$ CFU | 3/1 |
| Single Day | Single dose of $2 \times 10^{10}$ CFU | 3/1 |
| Single Day | Single dose of $2 \times 10^{11}$ CFU | 3/1 |
| Single Day | Single dose of $2 \times 10^{12}$ CFU | 3/1 |
| Single Day | Multiple dose of $2 \times 10^{12}$ CFU TID for 1 day | 3/1 |
| Single Day | Multiple dose of $5 \times 10^{11}$ CFU TID for 1 day | 3/1 |
| Single Day | Multiple dose of $1 \times 10^{12}$ CFU TID for 1 day | 3/1 |
| Multiple Day | Multiple dose of $2 \times 10^9$ CFU TID for 14 days | 6/2 |
| Multiple Day | Multiple dose of $2 \times 10^{11}$ CFU TID for 14 days | 6/2 |
| Multiple Day | Multiple dose of $5 \times 10^{11}$ CFU TID for 14 days | 6/2 |

Abbreviations: CFU = colony-forming unit(s); TID = ter in die (3 times daily)
Note: These preliminary data are based on a cutoff date of 30 October 2017 and may contain unresolved queries.

At the time of data cutoff, subjects in all cohorts had completed at least 72 hours of follow-up after the last dose of SYNB1020/placebo. Upon discharge from the inpatient unit, all subjects continued follow-up as an outpatient until 2 consecutive fecal samples were negative for SYNB1020 by polymerase chain reaction (PCR) analysis. At the time of cutoff, 8 subjects were continuing to be followed, and 44 subjects had completed the study. All subjects who completed study participation had fecal clearance within approximately 4 weeks following the last dose, and no subjects required treatment with antibiotics for positive fecal PCR. Four subjects discontinued the study prematurely due to mild to moderate treatment-emergent adverse events (TEAEs).

**Adverse Events**

As of 30 October 2017, there were no SAEs or deaths in the study. The most frequently reported TEAEs observed to date in Study SYNB1020-CP-001 were mild to moderate GI disorders, including nausea and vomiting. SYNB1020 was well tolerated in the single ascending dose (SAD) cohorts at single doses up to $5 \times 10^{11}$ CFU, which was determined to be the MTD. Six subjects (21.4%) experienced TEAEs in the SAD part of the study. Three subjects in the SAD part discontinued dosing due to TEAEs (moderate nausea and vomiting), including 2 subjects in the $2 \times 10^{12}$ CFU three times daily (TID) cohort and 1 subject in the $1 \times 10^{12}$ CFU TID cohort. Doses up to $5 \times 10^{11}$ CFU TID were well tolerated for up to 14 days in the MAD cohorts. Seven subjects (29.2%) experienced TEAEs in the MAD cohorts. One subject in the $5 \times 10^{11}$ CFU TID cohort discontinued dosing due to TEAEs (mild nausea and vomiting). All TEAEs leading to discontinuation in both the SAD and MAD cohorts occurred during the first day of dosing. Further safety data are presented in the Investigator's Brochure.

## 2.3. Rationale for the Study

Study SYNB1020-CP-002 represents the second clinical investigation of SYNB1020. Part 1 of the study comprises a sentinel cohort designed to establish the safety and tolerability of SYNB1020 in subjects with cirrhosis. Part 2 of the study is designed to evaluate the safety, tolerability, and pharmacodynamics (PD) of SYNB1020 compared with placebo in patients with hyperammonemia resulting from hepatic insufficiency. The dose of SYNB1020 to be administered in this study will be up to $5 \times 10^{11}$ CFU given TID immediately after meals for 6 days. The dose level is based on the MTD determined in the Phase 1 single and multiple-ascending dose study (Study SYNB1020-CP-001) in healthy adult volunteers (see Section 2.2.2.1). The duration of dosing (6 days) is based on the estimate that unmodified EcN requires 2 to 4 days to reach steady state.

Because SYNB1020 is a live biotherapeutic and is therefore susceptible to degradation by stomach acid, subjects enrolled in the study will receive a concomitant histamine-2 receptor (H2) inhibitor. Subjects will receive oral ranitidine (150 mg BID) beginning on Day -5 and for the duration of investigational product (IP) dosing (i.e., through Day 6). Ranitidine was selected for use as it is a widely used over-the-counter therapeutic with a well characterized safety profile and has demonstrated efficacy in increasing gastric pH. Subjects already on a proton pump inhibitor (PPI) will not be switched to ranitidine but will continue their prescribed PPI regimen for the duration of the study.

# 3. STUDY OBJECTIVES AND ENDPOINTS

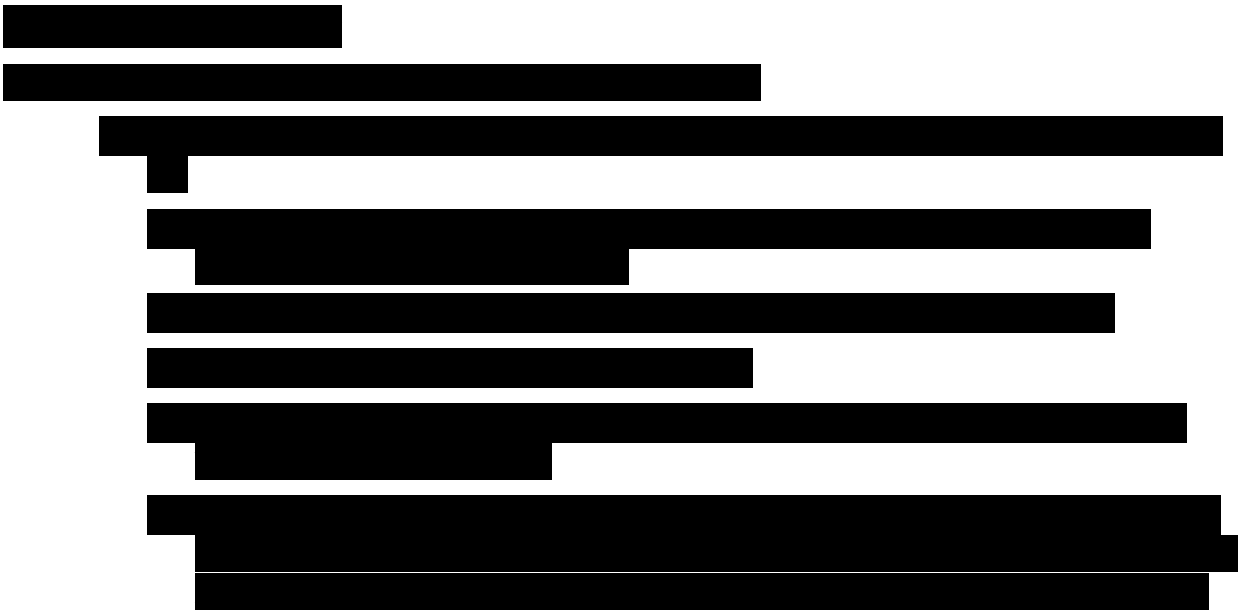
## 3.1. Primary

The primary objective is to evaluate the safety and tolerability of SYNB1020 following multiple doses in hepatic insufficiency and cirrhosis patients. The endpoints related to this objective include the nature and frequency of adverse events (AEs), measurement of vital signs and laboratory assessments (e.g., blood chemistry, complete blood count [CBC], liver function tests, international normalized ratio [INR], and urinalysis), and electrocardiograms (ECGs).

## 3.2. Secondary

Secondary objectives include evaluation of the following:

- SYNB1020 kinetics in feces (measured with qualitative and quantitative PCR fecal assays) following dosing
- Change from baseline of venous ammonia, including fasting and 24-hour area under the curve (AUC), following SYNB1020 dosing in subjects with hyperammonemia



# 4. STUDY DESIGN

## 4.1. Study Design Overview

This Phase 1b/2a, randomized, double-blinded placebo-controlled study will evaluate the safety, tolerability, and PD of SYNB1020 in hepatic insufficiency and cirrhosis patients, with dosing of the IP administered in an inpatient unit and subsequent outpatient follow-up for SYNB1020 clearance. Part 1 comprises a sentinel open-label cohort of 6 subjects with cirrhosis and Model for End-Stage Liver Disease (MELD) score <12 to establish the safety and tolerability of SYNB1020. Once the safety and tolerability have been established in Part 1, enrollment will be opened to subjects in Part 2, a randomized, double-blinded, placebo-controlled study in subjects with cirrhosis and hyperammonemia. Approximately 24 to 40 evaluable subjects are anticipated for enrollment in Part 2, with randomization proceeding in a 1:1 ratio to SYNB1020 and placebo.

In Part 1, eligible subjects will be admitted to an inpatient facility for a run-in diet, baseline assessments, IP administration, safety monitoring, and collection of blood, urine, and fecal samples for evaluation of safety, tolerability, and pharmacokinetic and PD evaluations.

In Part 2, subjects may be pre-screened for eligibility based on medical history and a single fasting spot venous ammonia measurement. Eligible subjects with elevated fasting spot venous ammonia will then undergo full screening within 7 days of pre-screening. Eligible subjects will be admitted to an inpatient facility for a run-in diet and 24-hour ammonia profile, and those with an elevated 24-hour ammonia AUC (>1.2 × ULN) will proceed with randomization, IP administration, safety monitoring, and collection of blood, urine, and fecal samples for pharmacokinetic and PD evaluations.

## 4.2. Study Dosing Regimens

### 4.2.1. Dose selection

Dosing is based on data from Study SYNB1020-CP-001, a Phase 1, dose-escalating, randomized, double-blinded study designed to determine the MTD of single and multiple doses of SYNB1020 in placebo-controlled cohorts of male and female healthy adult subjects. The dose administered in this study will be at or below the MTD of $5 \times 10^{11}$ CFU TID determined in Study SYNB1020-CP-001. The duration of dosing (6 days) was selected to be greater than the time to steady-state for unmodified EcN of 2 to 4 days. Preliminary data for the study are presented in Section 2.2.2.1.

### 4.2.2. Dietary Regimen and Study Supplements

All subjects will be placed on a weighed research diet calculated based on overall body weight (kg), age, and gender to meet each subject's caloric and protein (2 g/kg/day) requirements based on the dietary reference intakes (DRI).[20] This diet will be based on subject preferences as assessed during screening. Diets will include equal caloric and protein content on each day of inpatient monitoring. Subjects will be expected to consume the entirety of their meals and not consume any external foods for the duration of their inpatient study participation. Specifics regarding the diet can be found in the Diet Manual.

In addition, subjects will be administered an H2 inhibitor to reduce stomach acid. Oral ranitidine (150 mg) will be administered BID, 30 to 60 minutes before breakfast and in the evening, for the duration of the inpatient stay (Table 3). Subjects already on a PPI will not be switched to ranitidine but will continue their prescribed PPI regimen for the duration of the study.

### 4.2.3. Inpatient Monitoring and Investigational Product Administration

Each subject will be admitted to the inpatient unit after an overnight fast on the morning of Day -5. Beginning on Day -5, subjects will initiate a controlled diet and ranitidine. Days -5 through -1 will be a run-in phase to allow for dietary stabilization on the controlled diet. On Day -2, all subjects will undergo baseline 24-hour ammonia profile with 8 measurements (AUC), baseline clinical laboratory sampling and other safety measurements, FibroScan® measurement, and baseline HE grading [redacted]. In Part 1, all subjects will receive up to $5 \times 10^{11}$ CFU of SYNB1020 administered TID immediately after meals from Days 1 through 6 (including mid-day and evening doses on Day 6). Fasting ammonia level will be measured daily. On Day 5, subjects will undergo ammonia AUC assessment. On Day 6, subjects will undergo ammonia AUC assessment, repeat HE grading [redacted], as well as 24-hour urine and blood collection. Subjects will be monitored in the inpatient setting for at least 12 hours following completion of dosing, with discharge on Day 7 for clinically stable subjects. Stool samples will be collected daily during the inpatient period. Subjects will be followed for safety and SYNB1020 fecal clearance as outlined in Section 7.5. This cohort is open-label.

In Part 2, only subjects whose screening 24-hour AUC venous ammonia measurement is above 1.2 × the upper limit of normal (ULN) will undergo computer-generated randomization on Day -1 in a 1:1 ratio to receive either up to $5 \times 10^{11}$ CFU of SYNB1020 or matching placebo administered TID immediately after meals for 6 days. Other assessments will be the same as Part 1. Both subjects and investigators will be blinded to randomized IP assignment.

## 4.3. Stopping Rules

The Sponsor may stop this study at any time. Investigators will be notified by the Sponsor or its designee if the study is stopped. The occurrence of the following events will require that further enrollment in the study be stopped:

- Any suspected or proven invasive infection (e.g., sepsis or bacteremia) assessed as at least possibly related to the IP.
- Three or more subjects experience the same National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grade 3 AE assessed as at least possibly related to study treatment, or two or more subjects experience the same CTCAE grade 4 AE assessed as at least possibly related to the IP.
- Two or more subjects experience SAEs that are considered by the Investigator to be related to the IP.
- Death occurs at any time during the study and is considered by the Investigator to be related to the IP.

- Approximately 6% mortality is observed after 30 days following dosing. This is defined as ≥2 deaths observed in 24 subjects or ≥3 deaths observed in 40 subjects who have received the IP.
- The Investigator, Medical Monitor, or Sponsor determines that an event or current data warrant stopping the study.

The Sponsor's medical staff, the Contract Research Organization (CRO)'s pharmacovigilance physician and the Lead Investigator will review the data concerning these event(s), along with other available data. Based on the results of their investigation, the Sponsor's medical staff, the CRO's pharmacovigilance physician and the Lead Investigator will determine appropriate follow-up and decide whether the study should be stopped.

If the study is stopped, the events will be investigated, and the study will be closed to further enrollment. Subjects already participating in the study at the time the study is stopped will not receive further IP and will continue the follow-up schedule.

## 4.4. Safety Review Committee

A Safety Review Committee comprising the Lead Investigator, Medical Monitor, pharmacovigilance physician, and an external disease expert, as needed, will meet after the completion of Part 1 and ad hoc, as needed, to assess the safety and tolerability of SYNB1020 in subjects with cirrhosis, and to determine whether Part 2 can be conducted safely. The Part 1 safety evaluation will include any available data on AEs, laboratory measurements, vital signs, and ECGs up to 7 days after the last dose of SYNB1020. In Part 2, the Safety Review Committee will meet at predetermined intervals during the study and ad hoc, as needed, to conduct periodic reviews of interim safety data (see Section 9.7). Further details will be provided in a separate Safety Review Committee charter.

## 4.5. Duration of Study Participation

The maximum time of study participation for a subject is approximately 19 weeks (133 days).

### 4.5.1. Pre-screening Period in Part 2

The pre-screening period for subjects in Part 2 will be up to 7 days prior to initiation of full screening.

### 4.5.2. Screening Period

The screening period for all subjects will be up to 45 days prior to admission to the inpatient facility (i.e., Days -51 to -6).

### 4.5.3. Study Dosing

In Part 1, subjects will be dosed with SYNB1020 TID for 6 days. The total duration of inpatient study participation will be 12 days (Days -5 through 7).

In Part 2, subjects will be dosed with SYNB1020 or placebo TID for 6 days. The total duration of inpatient study participation will be 12 days (Days -5 through 7).

### 4.5.4. Post-dose Follow-up

One week (7 ± 1 days) after discharge from the inpatient unit, subjects will return for clinical evaluation and bring a 1-week post-dose fecal sample. Feces will continue to be collected weekly for the first 6 weeks following discharge and biweekly until a subject has a negative SYNB1020 fecal test for up to 10 weeks following completion of study dosing. Subjects who remain colonized with SYNB1020 after 10 weeks following the last dose will be treated with a 3-day course of oral ciprofloxacin (or other antibiotic for subjects allergic to ciprofloxacin), undergo a SYNB1020 fecal test 3 to 5 days following completion of antibiotic treatment, and be followed for AEs at least 7 days after completion of antibiotic treatment (see Section 7.5.2 for further details).

# 5. PATIENT POPULATION

## 5.1. Number of Subjects

Part 1: 6 evaluable subjects are anticipated for enrollment.

Part 2: Approximately 24 to 40 evaluable subjects, defined as subjects who complete all 3 ammonia 24-hour AUC intervals, are anticipated for enrollment.

## 5.2. Selection of Subjects

### 5.2.1. Eligibility Criteria

This study will comprise adult patients with hepatic insufficiency and cirrhosis. Patients will be eligible for enrollment regardless of gender or race/ethnicity.

#### 5.2.1.1. Inclusion Criteria in Part 1

Patients must meet all of the following inclusion criteria to be eligible for Part 1:

1. Male and female patients aged ≥ 18 to < 75 years.
2. Diagnosis of chronic (≥ 6 months), stable (no acute episodes of illness within the previous 2 months due to deterioration in hepatic function) hepatic insufficiency with features of cirrhosis due to any etiology.
3. MELD score <12 (see Appendix 5).
4. Able to produce at least 2 bowel movements per week on average without the assistance of laxatives.
5. Able and willing to voluntarily complete the informed consent process (patient or patient's representative).
6. Available for and agree to all study procedures, including inpatient monitoring and frequent blood sampling.
7. Male subjects who agree to use an acceptable method of contraception after informed consent, throughout the study, and for 90 days after the last dose of SYNB1020 or placebo.
8. Female subjects of non-childbearing potential: Either surgically sterile (defined as hysterectomy, bilateral oophorectomy, or tubal ligation/occlusion) or postmenopausal (defined as 12 months with no menses without an alternative medical cause and confirmed by follicle-stimulating hormone [FSH] in the range of menopause) confirmed at screening via medical history.
9. Screening laboratory evaluations within defined acceptable limits (Table 2), or judged to be not clinically significant by the investigator.

#### 5.2.1.2. Exclusion Criteria in Part 1

Patients meeting any of the following criteria are not eligible for Part 1 study enrollment:

1. Current or past HE of Grade 2 or higher using the West Haven Grading System (see Appendix 2) requiring hospitalization.
2. Child-Turcotte-Pugh score of > 9 (see Appendix 4).
3. History of liver transplant.
4. History of hepatocellular carcinoma; patients must have negative imaging result within 12 months prior to screening. (Exception: hepatocellular carcinoma that has been considered cured or in remission > 6 months prior to screening.)
5. Poorly controlled diabetes, or diabetes requiring insulin treatment.
6. Chronic obstructive pulmonary disease requiring the use of supplemental oxygen.
7. Visual or motor deficits leading to interference on psychometric testing.
8. Body mass index < 18.5 or ≥ 40 kg/m$^2$.
9. History of active or chronic passage of 3 or more loose stools per day within 4 weeks prior to screening.
10. Active inflammatory or irritable bowel disorder of any grade.
11. Active or prior history of GI bleeding within 8 weeks prior to screening, as confirmed by hospitalization-related event(s) or medical history of hematemesis or hematochezia.
12. Prior transjugular intrahepatic portosystemic shunt placement.
13. Prolonged QT interval corrected for heart rate (QTc) on screening ECG (defined as QTc > 500 ms)
14. Renal insufficiency (defined as serum creatinine >1.5 mg/dL).
15. Apart from chronic liver disease, any acute or chronic medical, surgical, psychiatric, or social condition including history of cerebrovascular disease (stroke, transient ischemic attack) or dementia, or laboratory abnormality that may increase the patient risk associated with study participation, compromise adherence to study procedures and requirements, confound interpretation of the safety, kinetics, or pharmacodynamics results, and, in the judgment of the investigator, make the patient inappropriate for enrollment.
16. Current or anticipated treatment with systemic (e.g., oral or intravenous) antibiotic (including rifaximin) within 4 weeks prior to screening through the final outpatient follow-up for any reason (e.g., infection or planned surgery, hospitalization, dental, or interventional studies) (Exception: topical antibiotics are allowed).
17. Current use of valproate, corticosteroids, or cytotoxic drugs.
18. Current or anticipated treatment for viral hepatitis within 3 months prior to screening through the final outpatient follow-up.
19. Current or prior use of laxatives (including lactulose) within 4 weeks prior to screening.

20. Current or prior use of probiotic supplements (excluding enriched foods such as yogurt) within 4 weeks prior to screening.
21. Any condition, prescription medication, or over-the-counter product that may possibly affect absorption of medications or nutrients (e.g., celiac disease, gastrectomy, bypass surgery, ileostomy).
22. Alcohol intake of more than two units per week within 1 month prior to screening.
23. Dependence on drugs of abuse, use of any opiods (except stable regimen of prescription drugs).
24. Administration or ingestion of an investigational drug within 8 weeks or 5 half-lives, whichever is longer, prior to screening or current enrollment in an investigational study.
25. History of allergy to ranitidine, or intolerance to any of the excipients (glycerol, CVS Health Easy Fiber).

#### 5.2.1.3. Inclusion Criteria in Part 2

Patients must meet all of the following inclusion criteria to be eligible for Part 2:

1. Male and female patients aged ≥ 18 to < 75 years.
2. Diagnosis of chronic (≥ 6 months), stable (no acute episodes of illness within the previous 2 months due to deterioration in hepatic function) hepatic insufficiency with features of cirrhosis due to any etiology.
3. Fasting venous ammonia > ULN as defined by the site laboratory required for admission; ammonia AUC >1.2 × ULN required for randomization.
4. Able and willing to voluntarily complete the informed consent process (patient or patient's representative).
5. Available for and agree to all study procedures, including inpatient monitoring and frequent blood sampling.
6. Male subjects who agree to use an acceptable method of contraception after informed consent, throughout the study, and for 90 days after the last dose of SYNB1020 or placebo.
7. Female subjects of non-childbearing potential: Either surgically sterile (defined as hysterectomy, bilateral oophorectomy, or tubal ligation/occlusion) or postmenopausal (defined as 12 months with no menses without an alternative medical cause and confirmed by FSH in the range of menopause) confirmed at screening via medical history.
8. Screening laboratory evaluations within defined acceptable limits (Table 2), or judged to be not clinically significant by the investigator.

#### 5.2.1.4. Exclusion Criteria in Part 2

Patients meeting any of the following criteria are not eligible for Part 2 study enrollment:

1. Current or past HE of Grade 2 or higher using the West Haven Grading System (see Appendix 2) requiring hospitalization.

2. Child-Turcotte-Pugh score of > 9 (see Appendix 4).
3. MELD score >20 (see Appendix 5).
4. History of liver transplant.
5. History of hepatocellular carcinoma; patients must have negative imaging result within 12 months prior to screening. (Exception: hepatocellular carcinoma that has been considered cured or in remission > 6 months prior to screening.) Imaging may be obtained during the screening period.
6. Poorly controlled diabetes or significant changes to diabetes medications within 6 weeks prior to Day -5.
7. Chronic obstructive pulmonary disease requiring the use of supplemental oxygen.
8. Body mass index < 18.5 or ≥ 40 $kg/m^2$.
9. History of active or chronic passage of 3 or more loose stools per day within 4 weeks prior to screening.
10. Active inflammatory or irritable bowel disorder of any grade.
11. Active or prior history of GI bleeding within 8 weeks prior to screening, as confirmed by hospitalization-related event(s) or medical history of hematemesis or hematochezia.
12. Prior transjugular intrahepatic portosystemic shunt placement.
13. Prolonged QTc on screening ECG (defined as QTc > 500 ms).
14. Renal insufficiency (defined as serum creatinine >1.5 mg/dL).
15. Apart from chronic liver disease, any acute or chronic medical, surgical, psychiatric, or social condition including history of cerebrovascular disease (stroke, transient ischemic attack) or dementia, or laboratory abnormality that may increase the patient risk associated with study participation, compromise adherence to study procedures and requirements, confound interpretation of the safety, kinetics, or pharmacodynamics results, and, in the judgment of the investigator, make the patient inappropriate for enrollment.
16. Current or anticipated treatment with systemic (e.g., oral or intravenous) antibiotic (including rifaximin) within 4 weeks prior to Day -5 through the final outpatient follow-up for any reason (e.g., infection or planned surgery, hospitalization, dental, or interventional studies) (Exception: topical antibiotics are allowed).
17. Current use of valproate, corticosteroids, or cytotoxic drugs.
18. Current or anticipated treatment for viral hepatitis within 3 months prior to Day -5 through the final outpatient follow-up.
19. Current or prior use of lactulose within 2 weeks prior to Day -5.
20. Current or prior use of probiotic supplements (excluding enriched foods such as yogurt) within 4 weeks prior to Day -5.

21. Any condition, prescription medication, or over-the-counter product that may possibly affect absorption of medications or nutrients (e.g., celiac disease, gastrectomy, bypass surgery, ileostomy).
22. Alcohol intake of more than two units per week within 1 month prior to screening.
23. Dependence on drugs of abuse, use of any opiods (except stable regimen of prescription drugs).
24. Administration or ingestion of an investigational drug within 8 weeks or 5 half-lives, whichever is longer, prior to screening or current enrollment in an investigational study.
25. History of allergy to ranitidine, or intolerance to any of the excipients (glycerol, CVS Health Easy Fiber).

**Table 2: Laboratory Exclusion Criteria**

| Laboratory measure | Exclusion criterion |
|---|---|
| White blood cell count | $< 1\times10^9$/L |
| Hemoglobin | < 8.5 g/dL |
| Platelet count | < 35,000 /μL |
| aPTT | > 1.5 × ULN |
| PT | > 4.0 sec prolongation from ULN |
| INR | > 1.7 |
| Serum creatinine | >1.5 mg/dL |
| Total bilirubin | > 50 μmol/L or > 3 mg/dL, unless diagnosed with Gilbert's syndrome |
| Serum Albumin | < 2.8 g/L |
| AST and/or ALT | > 3 × ULN |
| P-sodium | < 132 or > 148 mmol/L |
| P-potassium | < 3.2 or > 5.3 mmol/L |
| QTc | > 500 ms |

Abbreviations: aPTT = activated partial thromboplastin time; ALT = alanine aminotransferase; AST = aspartate aminotransferase; INR = international normalized ratio; PT = prothrombin time; QTc = QT interval corrected for heart rate; ULN = upper limit of normal

# 6. INVESTIGATIONAL MEDICAL PRODUCT AND CONCOMITANT MEDICATIONS

## 6.1. Investigational Product Supply and Administration

Within this protocol, IP refers to SYNB1020 and/or placebo.

### 6.1.1. SYNB1020

#### 6.1.1.1. Manufacturing and Formulation

SYNB1020 investigational drug product is formulated in 15% glycerol/5% trehalose/10 mM 3-(N-morpholino) propanesulfonic acid (MOPS), pH 7.0. The formulated SYNB1020 drug product will be further diluted in a taste-masking solution for PO.

#### 6.1.1.2. Packaging and Labeling

SYNB1020 is packaged as a frozen liquid suspension in 5-mL cryogenic vials (5-mL/vial). The following is a sample vial label (final study label may vary):

Investigational Protocol Number: SYNB1020-CP-002
Lot Number: XXXX
Description: SYNB1020 at $10^{11}$ CFU/mL
Dosing Instructions: Oral dose
Retest Date: Month Year
Store at < -65˚C
"Caution: New Drug – Limited by Federal (or United States) law to investigational use."
SYNLOGIC INC
Manufactured by: Paragon Bioservices
801 West Baltimore Street, Suite 401
Baltimore, MD 21201
Contact Information: XXX-XXX-XXXX

#### 6.1.1.3. Shipping, Storage, and Handling

Labeled, packaged SYNB1020 will be shipped to the study center(s) by the CRO (Parexel). SYNB1020 should be stored at < -65°C.

Detailed instructions for the storage, handling, and administration of IP will be provided in the Pharmacy Manual and may override instructions provided in the following protocol sections.

#### 6.1.1.4. Dispensing and Administration

The manufactured SYNB1020 drug product will have a pre-specified concentration. To obtain appropriate doses, vial(s) of concentrated drug product will be thawed and appropriately diluted in a chilled formulation buffer and masking solution.

### 6.1.2. Placebo

Placebo will contain only 100 mL of a chilled masking solution.

### 6.1.3. Ranitidine

Ranitidine will be obtained from a commercial source and supplied to study sites as 150-mg tablets for oral administration. Ranitidine should be stored in accordance with the prescribing information (see Appendix 1).

## 6.2. Accountability and Dosing Compliance

All doses of IP will be administered in the clinic by trained study personnel during inpatient hospitalization. A temperature mechanism will be included with the IP to ensure the temperature has not been compromised.

## 6.3. Concomitant Medications

Concomitant medications can be administered at the Investigator's discretion to conform to standard practice during the dosing period. Overall, SYNB1020 has a low likelihood for eliciting clinically relevant drug-drug interactions; however, Investigators should use caution when prescribing concomitant-medications, as this compound has not been extensively investigated in the clinical setting. Investigators should contact the Medical Monitor when they are unsure as to whether a drug should be prescribed to a subject. All concomitant medications and dietary supplements will be recorded in the electronic case report forms (eCRFs) using generic drug names when possible.

### 6.3.1. Prohibited Therapies and Substances

Therapies and substances that are prohibited at screening and during study participation are outlined in the study eligibility criteria (Section 5.2.1).

# 7. STUDY PROCEDURES AND ASSESSMENTS

Before recruitment of subjects into the study, written Institutional Review Board (IRB) approval of the protocol, informed consent, and any additional patient information must be obtained.

The various assessments that will be conducted during this study are summarized in Table 3 (Schedule of Events).

Detailed instructions regarding sample collection and handling, as well as all laboratory procedures, will be provided to the study site in separate study manuals. Site-specific handling instructions for hematology and clinical chemistry samples should be followed.

## 7.1. Screening

Subjects who fail screening due to a transient cause or missing information are eligible for re-screening.

### 7.1.1. Part 1

Screening procedures must be performed to determine eligibility for enrollment in Part 1 within 45 days prior to admission to the inpatient facility. If a particular assessment is repeated, the results obtained closest to the first dose of IP should be used to assess eligibility.

During screening, a unique number will be assigned to each subject who signs informed consent for the study. Once a subject is enrolled in the study, s/he will only be identified by the assigned study number.

At the site, the Investigator will maintain a log for all screened subjects (including subjects who failed screening after signing informed consent) and all enrolled subjects.

### 7.1.2. Part 2

#### 7.1.2.1. Pre-screening

Subjects may be pre-screened for eligibility in Part 2 based on medical history and a single spot fasting venous ammonia measurement. Subjects passing the pre-screen will undergo full evaluation and screening procedures within 7 days.

#### 7.1.2.2. Screening

Screening procedures must be performed to determine eligibility for enrollment in Part 2 within 45 days prior to admission to the inpatient facility. If a particular assessment is repeated, the results obtained closest to the first dose of IP should be used to assess eligibility, with the exception of fasting venous ammonia, which will not be repeated if measured at pre-screening.

During screening, a unique number will be assigned to each subject who signs informed consent for the study. If a subject meets all the inclusion criteria and none of the exclusion criteria, including ammonia AUC after the run-in period, and is selected to participate in the study, s/he will receive a randomization number. Once a subject is enrolled in the study, s/he will only be identified by the assigned randomization number.

The Investigator or designee is responsible for verifying that the subject is eligible before requesting a randomization number. At the site, the Investigator will maintain a log for all screened subjects (including subjects who failed screening after signing informed consent) and all enrolled subjects.

Subjects must be dosed within 3 calendar days of randomization.

## 7.2. Safety Assessments

### 7.2.1. Adverse Events

Adverse events will be assessed continuously by direct observation and subject interviews. The severity of AEs will be evaluated using the NCI CTCAE, version 4.03.[21] All AEs occurring from the time a subject signs informed consent through the safety follow-up period will be recorded, regardless of causal assessment to SYNB1020/placebo. After the end of the follow-up period, only AEs that are considered related to IP will be recorded, if clinically indicated.

### 7.2.2. Medical History

Past and present medical history will be recorded. Any ongoing condition and signs and symptoms observed prior to the initiation of study dosing, including bowel habits, should be recorded as medical history.

### 7.2.3. Vital Signs

Semi-supine vital signs (systolic blood pressure, diastolic blood pressure, pulse, and body temperature) will be collected at screening and at pre-specified pre- and post-dose time points during inpatient monitoring (see Table 3). Subjects are required to remain in the semi-supine position for at least 5 minutes prior to obtaining vital signs.

### 7.2.4. Physical Examination

Complete physical examinations will be performed by trained medical personnel during screening and baseline (Day -5), with symptom-directed physical examinations performed at subsequent time points (see Table 3). Any abnormal findings will be recorded as AEs.

### 7.2.5. FibroScan

A FibroScan (vibration-controlled transient elastrography) will be performed at baseline to quantify liver fibrosis.

### 7.2.6. Clinical Laboratory Measurements

The following clinical safety laboratory tests will be performed at the time points specified in Table 3: CBC with differential, prothrombin time (PT)/activated partial thromboplastin time (aPTT), serum chemistry including liver function tests (LFTs), and urinalysis. These tests will include the parameters necessary for calculation of the MELD score (i.e., serum bilirubin, serum creatinine, and INR; see Appendix 5). Screening for infectious diseases and drugs of abuse will also be performed prior to study dosing. At screening, if non-childbearing potential status is equivocal based on medical history, FSH will be assessed in selected females to confirm post-menopausal status.

Screening results will be assessed by the Investigator for inclusion of subjects in the study. Additionally, unscheduled clinical laboratory tests can 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.

### 7.2.7. Venous Ammonia

Ammonia samples will be analyzed locally. Samples for plasma ammonia will be obtained from a free-flowing venous blood sample. Plasma should be separated immediately after collection, and analysis must be performed within 60 minutes. Refer to the Laboratory Manual for further details.

The normal reference range of ammonia at each site will be defined based on approximately 30 healthy volunteers devoid of any chronic or acute medical conditions and medications with normal liver enzymes. Ammonia sampling, sample processing and analysis will be performed as defined in this protocol and the coordinating laboratory manual. The 95th percentile will be used as the ULN for each site.

### 7.2.8. Electrocardiograms

A semi-supine single 12-lead ECG will be performed at screening, baseline, on Day 4, and at discharge. Electrocardiogram parameters to be evaluated include the RR, QT, QRS, and PR intervals. In addition, Fridericia's formula should be used to calculate the QT interval corrected for heart rate (QTcF).

### 7.2.9. Blood and Urine Cultures/Polymerase Chain Reaction

Blood and urine cultures and/or PCR will be performed as needed according to standard clinical practice if clinical presentation warrants. This may include, but is not limited to, cases in which subjects develop persistent (> 24 hours) fever, chills, night sweats, or other symptoms suggestive of local or systemic clinical infection. SYNB1020 is a thymidine auxotroph and requires sufficient concentration of thymidine in the cell culture media. Refer to the Laboratory Manual for further details.

### 7.2.10. Hepatic Encephalopathy Severity Grading

The severity of HE on study will be evaluated at the pre- and postdose time points specified in Table 3 using the West Haven Grading System, which ranges from Grade 0 to Grade 4 depending on the extent of a subject's impairment of consciousness, intellectual function, and/or behavior (see Appendix 2).

[redacted]

### 7.2.12. Cirrhosis Severity Grading

The severity of cirrhosis will be assessed using the Child-Turcotte-Pugh score, which reflects a composite score incorporating HE grade, extent of ascites, and bilirubin, albumin, and INR values (see Appendix 4) and MELD score calculated based on bilirubin, creatinine, and INR values (see Appendix 5).

## 7.3. Microbiotic-Kinetic Assessments

Feces from each voiding of the day will be collected at the pre- and postdose time points specified in Table 3 for analysis of SYNB1020 transit through the GI tract. In Part 1, the 24-hour fecal sample will be weighed, homogenized, and a sample analyzed for SYNB1020 by qPCR. In Part 2, a fecal sample from the first void of the day will be collected using a collection kit. For full details on sample collection and preparation for shipment, refer to the study-specific laboratory manual.

## 7.4. Pharmacodynamic Assessments

In addition to the analyses specified in the following sections, serum, plasma, urine, and feces will be collected and stored for exploratory analyses, which may include, but are not limited to, microbiome analyses, fecal arginine measurements, and an ex vivo fecal ammonia consumption studies. For full details on sample collection, refer to the study-specific laboratory manual. A single blood sample may be collected at pre-screening. Urine and blood samples will be collected at screening and at the pre- and postdose time points specified in Table 3. The following laboratory measurements will be performed to evaluate the preliminary pharmacodynamics of SYNB1020:

- Plasma ammonia (morning fasting and 24-hour profile to calculate AUC)
  - Samples for venous ammonia will be drawn relative to meal times (breakfast, lunch, and dinner) according to the following schedule. Following an overnight fast, subjects will have a baseline sample drawn for ammonia at 0 hours (before breakfast) and will be given a high-protein shake for breakfast, followed by IP administration on dosing days. Subsequently, samples for venous ammonia will be drawn on:
    - Day -2: +2 hours, +4 hours (before lunch), +6 hours, +9 hours (before dinner), +11 hours, +14 hours, +18 hours, and +24 hours (before breakfast on Day -1).
    - Day 5: +2 hours, +3 hours, +4 hours (before lunch), +5 hours, +6 hours, +9 hours (before dinner), +11 hours, +14 hours, +18 hours, and +24 hours (before breakfast on Day 6).
    - Day 6: +2 hours, +4 hours (before lunch), +6 hours, +9 hours (before dinner), +11 hours, +14 hours, +18 hours, and +24 hours (before breakfast on Day 7).

[redacted]

[redacted]

- Bristol stool form scale (BSFS) for each bowel movement[22]

[redacted]

## 7.5. Follow-up Assessments

### 7.5.1. End-of-study/Discharge Visit

At least 12 hours after completion of the dosing period (i.e., on Day 7 for subjects who continue IP dosing per protocol), an EOS/Discharge visit will be performed. For subjects who discontinue study dosing prematurely, the EOS/Discharge Visit should be completed within 24 hours of discontinuation, and every effort should be made to perform the procedures specified in (Table 3).

### 7.5.2. Follow-up After Completion of Study Dosing

One week (7 ± 1 days) after discharge from the inpatient unit, subjects will return for clinical evaluation and bring a 1-week post-dose fecal sample. Subjects will be provided diary cards for collection of AE data. Feces will continue to be collected weekly for the first 6 weeks following discharge and biweekly until a subject has a negative SYNB1020 fecal test for up to 10 weeks following completion of study dosing. Subjects who remain colonized with SYNB1020 after 10 weeks following the last dose will be treated with a 3-day course of oral ciprofloxacin. Subjects who are allergic to ciprofloxacin can be treated with antibiotics based on reported sensitivity at the investigator's discretion. Refer to the SYNB1020 Investigator's Brochure for further details. A SYNB1020 fecal test will be performed 3 to 5 days following completion of antibiotic treatment solely for research purposes and will not guide subject follow-up decision making. An AE assessment will be performed by telephone at least 7 days after completion of antibiotic treatment. If no AEs are reported, subjects will not require any further follow-up and will be considered to have completed the study requirements. If AEs are reported, subjects will be advised to return to the inpatient facility for a comprehensive physical examination, laboratory assessment, and AE causality determination.

## 7.6. Sample Retention for Exploratory Analyses

Blood, urine, and fecal samples may be stored for future analyses related to metabolism and microbiome evaluations for up to 5 years. Any unused samples will be destroyed.

## Table 3: Schedule of Events

| Study Period | Optional Pre-Screening | Screening | Inpatient Monitoring | | | | | | | | | | Follow-up[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **Within 7 days of Screening** | **-51 to -6** | **-5** | **-4** | **-3** | **-2** | **-1** | **1** | **2-4** | **5** | **6** | **7 EOS/Discharge Visit[b]** | **14 Follow-up Visit** | **Up to 70 days after last dose** |
| Informed consent for pre-screening | ● | | | | | | | | | | | | | |
| Informed consent | | ● | | | | | | | | | | | | |
| Medical history | ● | ●[c] | ● | | | | | | | | | | | |
| Detection of elevated portal hypertension | | ●[d] | | | | | | | | | | | | |
| Imaging to rule out hepatocellular carcinoma | | ●[d] | | | | | | | | | | | | |
| Physical examination | | ● | ● | | | | | | | | | ●[e] | ●[e] | |
| FibroScan | | | | | | ● | | | | | | | | |
| Screening for drugs of abuse (urine drug screen) | | ● | ● | | | | | | | | | | | |
| Screening for infectious disease (HIV/Hepatitis B, C serology) | | ● | | | | | | | | | | | | |
| Laboratory tests (CBC, LFTs, blood chemistry, urinalysis, PT/aPTT) | | ● | | | | ● | | | ●[f] | | ● | ● | | |
| FSH (in females if non-childbearing potential status is equivocal based on medical history) | | ● | | | | | | | | | | | | |
| Record concomitant medications | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | |
| Adverse event reporting | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | |
| Vital signs[g] | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | |
| Electrocardiogram | | ● | | | | ● | | | ●[f] | | | ● | | |
| Fasting spot venous ammonia | ● | ●[h] | | | | ● | | ● | ● | ● | ● | ● | | |
| Admit to inpatient facility (in the morning after an overnight fast) | | | ● | | | | | | | | | | | |
| Required inpatient stay | | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | | |
| Subject randomization (Part 2 only) | | | | | | | ● | | | | | | | |
| Dietary control[i] | | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | | |

| Study Period | Optional Pre-Screening | Screening | Inpatient Monitoring | | | | | | | | | | Follow-up[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **Within 7 days of Screening** | **-51 to -6** | **-5** | **-4** | **-3** | **-2** | **-1** | **1** | **2-4** | **5** | **6** | **7 EOS/Discharge Visit[b]** | **14 Follow-up Visit** | **Up to 70 days after last dose** |
| Administer H2 inhibitor (ranitidine 150 mg BID 30-60 minutes before breakfast and the evening snack)[j] | | | ● | ● | ● | ● | ● | ● | ● | ● | ● | | | |
| Administer assigned IP (SYNB1020 or placebo, TID) with breakfast, lunch, and dinner[k] | | | | | | | | ● | ● | ● | ● | | | |
| 24-hour profile for venous ammonia[l] | | | | | | ● | | | | ● | ● | | | |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| Fecal SYNB1020 PCR[n] | | | ● | | | | | ● | ● | ● | ● | ● | ● | ● |
| [redacted] | | | | | | ■ | | | | | ■ | | | |
| Hepatic encephalopathy grading (West Haven grade) | | | | | | ● | | | | | ● | | | |
| Child-Turcotte-Pugh score | | ● | | | | ● | | | | | ● | | | |
| Model for End-Stage Liver Disease score | | ● | | | | ● | | | ● [f] | | ● | | | |
| BSFS for each bowel movement collected | | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | | |
| Plasma collection for exploratory analyses | | | | | | | ● | | | | ● | | | |
| Urine collection for exploratory analyses | | | | | | | ● | | | | ● | | | |
| Review of adverse event diary card | | | | | | | | | | | | | | ● |

Abbreviations: aPTT = activated partial thromboplastin time; BID = twice daily; BSFS = Bristol stool form scale; CBC = complete blood count; EOS = End of Study; FSH = follicle-stimulating hormone; h = hour(s); H2 = histamine-2 receptor; HIV = human immunodeficiency virus; [redacted] IP = investigational product; LFT = liver function test; PCR = polymerase chain reaction; PPI = proton pump inhibitor; PT = prothrombin time; TID = 3 times daily; [redacted]

[a] One week (7± 1 days) after discharge from the inpatient unit, subjects will return for clinical evaluation and bring a 1-week post-dose fecal sample. Feces will continue to be collected weekly for the first 6 weeks following discharge and biweekly until a subject has a negative SYNB1020 fecal test for up to 10 weeks following completion of study dosing. Subjects who remain colonized with SYNB1020 after 10 weeks following the last dose will be treated as described in Section 7.5.2.

[b] At least 12 hours after completion of the dosing period (i.e., on Day 7 for subjects who continue IP dosing per protocol), an EOS/Discharge visit will be performed. For subjects who discontinue study dosing prematurely after having received at least one dose of SYNB1020/placebo, the EOS/Discharge Visit should be completed within 24 hours of discontinuation.
[c] If not obtained during pre-screening.
[d] Procedures to detect evidence of elevated portal hypertension (i.e., FibroScan to measure liver stiffness, endoscopy to determine the presence of abdominal or esophageal varices, imaging to detect splenomegaly, or ascites) and imaging to rule out hepatocellular carcinoma may be performed during screening if necessary to determine eligibility as outlined in Section 5.2.1.3.
[e] Symptom-driven physical examinations will be performed on Days 7 and 14.
[f] Day 4 only.
[g] Vital signs will be collected every 8 hours during the dosing period (Days 1 through 6).
[h] Fasting venous ammonia will not be repeated at screening if measured at pre-screening.
[i] Meal times for breakfast, lunch, and dinner should be the same every day ± 30 minutes, unless otherwise specified.
[j] Subjects already on a PPI regimen will not be switched to ranitidine but will continue their prescribed PPI regimen for the duration of the study.
[k] In Part 1, all subjects receive open-label IP. In Part 2, subjects are randomized to either SYNB1020 or placebo. IP will be administered immediately following a meal.
[l] Samples for venous ammonia will be drawn relative to meal times according to Table 4. Following an overnight fast, subjects will have a baseline sample drawn for ammonia at 0h (before breakfast) and will be given a high-protein shake for breakfast, followed by IP administration on dosing days. Samples for venous ammonia will be drawn at +2h, +4h (before lunch), +6h, +9h (before dinner), +11h, +14h, +18h, and +24h the following morning (before breakfast). Two additional samples will be collected on Day 5 at +3h and +5h [redacted]
[n] One baseline fecal sample is required to be collected prior to SYNB1020 dosing (i.e., any time from Day -5 through Day 1). Feces will continue to be collected weekly for the first 6 weeks following discharge and biweekly (every other week) until a subject has a negative SYNB1020 fecal test for up to 10 weeks following completion of study dosing. Subjects who remain colonized with SYNB1020 after 10 weeks following the last dose will be treated with a 3-day course of oral ciprofloxacin, or other antibiotics. On Day 7, if no void has occurred by the time all other assessments have been completed, the subject may be discharged without a fecal sample.

## Table 4: Schedule of Plasma Sampling for 24-Hour Ammonia [redacted]

| Study Day<br>Activity[a] | Time of Day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day -2** | **0 hours** | **+2 hours** | **+3 hours** | **+4 hours** | **+5 hours** | **+6 hours** | **+9 hours** | **+11 hours** | **+14 hours** | **+18 hours** | **+24 hours** |
| Plasma draw for 24-hour ammonia | ● | ● | | ● | | ● | ● | ● | ● | ● | ● |
| [redacted] | [redacted] | [redacted] | | [redacted] | | [redacted] | | | | | |
| Breakfast | ● | | | | | | | | | | |
| Lunch | | | | ● | | | | | | | |
| Dinner | | | | | | | ● | | | | |
| **Day 5** | | | | | | | | | | | |
| Plasma draw for 24-hour ammonia | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Breakfast | ● | | | | | | | | | | |
| Give IP | ● | | | ● | | | ● | | | | |
| Lunch | | | | ● | | | | | | | |
| Dinner | | | | | | | ● | | | | |
| **Day 6** | | | | | | | | | | | |
| Plasma draw for 24-hour ammonia | ● | ● | | ● | | ● | ● | ● | ● | ● | ● |
| [redacted] | [redacted] | [redacted] | | [redacted] | | [redacted] | | | | | |
| Breakfast | ● | | | | | | | | | | |
| Give IP | ● | | | ● | | | ● | | | | |
| Lunch | | | | ● | | | | | | | |
| Dinner | | | | | | | ● | | | | |

Abbreviations: IP = investigational product
[a] Plasma draws occur before meals and meals occur before IP administration, as applicable.

# 8. STUDY DISCONTINUATION

## 8.1. Sponsor Discontinuation Criteria

This study may be discontinued at any time due to safety concerns (including, but not limited to, the stopping rules described in Section 4.3), 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 reason(s) for early termination. The Investigator must inform the IRB promptly and provide the reason(s) for the termination.

## 8.2. Study Discontinuation for Individual Subjects

Subjects must discontinue further IP administration and withdraw from the study for the following reasons:

- Withdrawal of consent;
- Investigator decision;
- Significant noncompliance with protocol procedures;
- >1 missed dose of investigational product
- Necessity for treatment with systemic antibiotics;
- Clinically significant allergy or hypersensitivity reaction to SYNB1020;
- Development of any of the following toxicities:
  - AE of Grade 3 or greater severity according to the NCI CTCAE or life-threatening AE if not covered by CTCAE;
  - Episode of clinically overt HE (West Haven grade 2 or higher; see Appendix 2)
  - QTc interval of > 520 msec or an increase from baseline of > 60 msec confirmed in a repeat test after at least 20 minute;
  - Any of the following laboratory abnormalities:
    - Grade 3 liver enzyme abnormalities and 10-fold ULN for those with normal values at baseline OR a 5-fold increase or 10-fold ULN for those with abnormal values at baseline, whichever is lower;
    - Hemoglobin levels of < 8 g/dL confirmed in a repeat test or requiring blood transfusion;
    - Platelet count of < 20,000/mL confirmed in a repeat test;
    - eGFR < 30 mL/min/1.73m$^2$ confirmed in a repeat test;
    - PT > 6.0 sec over ULN or INR level of > 2.3 confirmed in a repeat test;
    - Bilirubin > 5 × ULN confirmed in a repeat test;

- Any other laboratory abnormality of Grade 3 or great severity not covered above and not present at baseline, confirmed in a repeat test.

Subjects 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 subject withdraws consent, the date and stated reason for consent withdrawal should be documented. Subject data collected up to the date of consent withdrawal will be included in the analyses.

Subjects who withdraw from the study prior to receiving SYNB1020/placebo are not required to complete the safety follow-up period. Subjects who receive at least one dose of IP and withdraw from the study are required to complete the tests and evaluations for the EOS/Discharge Visit at the time of withdrawal. Subjects who discontinue study treatment after the first administration of SYNB1020/placebo and withdraw from the study will not be allowed to enroll again at a later date. If a subject misses a dose of IP for any reason, the subject should continue with the study and take the next dose at the time specified in Table 3. A subject who misses more than one dose of IP should be withdrawn from the study. If a subject who withdraws from the study has an ongoing AE, every effort will be made to follow the AE until the event has resolved, stabilized, or returned to baseline status. The Sponsor must be notified of all study withdrawals within 24 hours.

# 9. ADVERSE EVENTS

## 9.1. Adverse Event

An AE is any untoward medical occurrence, including the exacerbation of a pre-existing condition, in a subject administered a pharmaceutical product regardless of causality.

### 9.1.1. Assessment of Severity

The severity rating of an AE refers to its intensity. The severity of each AE will be categorized using the NCI CTCAE, version 4.03.[21] 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

### 9.1.2. 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 SYNB1020/placebo, and de-challenge or re-challenge.

**Yes** (possibly, probably, or definitely related): there is a reasonable possibility that SYNB1020/placebo caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of SYNB1020/placebo.
- The event could not be reasonably attributed to the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- The event follows a known pattern of response to SYNB1020.
- 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).

**No** (unlikely, probably not related, or definitely not related): there is no reasonable possibility that the IP caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of SYNB1020/placebo
- The event could be reasonably attributed to the known characteristics of the subject's clinical state, concurrent illness, environmental or toxic factors, or other modes of therapy administered to the subject.
- The event does not follow a known pattern of response to SYNB1020.
- The event does not disappear or decrease on cessation or reduction in dose(ing), and it does not reappear or worsen when dosing is resumed.

## 9.2. Serious Adverse Event

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 subject 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 subject or may require intervention to prevent one of the other outcomes described above.

### 9.2.1. 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 subject 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.

- Pre-planned 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 SYNB1020 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:
  - Subjects 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.

### 9.2.2. 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 as necessary, of any AE associated with IP administration or study procedures that is a serious, unexpected, suspected adverse reaction (SUSAR) or any finding from tests in laboratory animals that suggests a significant risk for human subjects, 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.

## 9.3. Procedures for Treating and Reporting Special Safety Events

### 9.3.1. Infection (Suspected or Confirmed)

Subjects who demonstrate any symptoms, signs, and/or laboratory assessments suggestive of possible infection during the dosing or follow-up periods should be assessed for infection, including blood and urine evaluation (culture and/or PCR, see Section 7.2.9). Samples of all available body fluids should be obtained for PCR assessment prior to initiating broad spectrum antibiotic treatment guided by the subject's clinical condition and local standard of care; however, administration of antibiotics should not be significantly delayed to allow for collection (e.g., of feces). Subjects who require systemic antibiotic treatment should discontinue IP dosing and the event should be reported to the Sponsor within 24 hours. SYNB1020 is a Gram-negative bacteria with no antibiotic resistance genetic markers. Antibiotic sensitivity testing has been performed as described in the Investigator's Brochure and can be used to guide treatment if infection is suspected.

### 9.3.2. Spontaneous Bacterial Peritonitis

Spontaneous bacterial peritonitis (SBP) is considered an adverse event of special interest. Suspected or confirmed cases of SBP should be diagnosed and treated per local standard of care. Samples of all available body fluids should be obtained for PCR assessment prior to initiating broad spectrum antibiotic treatment. Subjects who develop SBP should discontinue IP dosing and the event should be reported to the Sponsor within 24 hours.

### 9.3.3. Pregnancy

If a subject becomes pregnant while participating in this study, information on the pregnancy and outcome will be requested. If a partner of a participating subject becomes pregnant while their partner is participating in the study or 90 days following their participation, information will be requested on the pregnancy and outcome.

### 9.3.4. Hepato-Renal Syndrome

Hepato-renal syndrome (HRS) is considered an adverse event of special interest. Suspected or confirmed cases of HRS should be diagnosed and treated per local standard of care. Subjects who develop HRS should discontinue IP dosing and the event should be reported to the Sponsor within 24 hours.

## 9.4. 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 subject signs informed consent through the safety follow-up period must be recorded on the eCRF. Subjects will be provided with diary cards for recording AEs during follow-up period after discharge from the inpatient unit.

All SAEs, regardless of relationship to SYNB1020/placebo, must be reported to the Sponsor within 24 hours of the Investigator's knowledge. This should be done by faxing the completed SAE Report Form to the Sponsor at the number provided on the SAE Report Form. After the final follow-up visit, only SYNB1020/placebo-related AEs/SAEs need to be collected and reported to the Sponsor, if clinically indicated.

Investigators must follow subjects with AEs/SAEs until event resolution or stabilization, withdrawal of consent, subject loss to follow-up, or death, whichever occurs first.

## 9.5. Pregnancy

Only females of non-childbearing potential are eligible for study participation.

## 9.6. 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 range(s). All abnormal values assessed to be of clinical concern and at least possibly related to SYNB1020/placebo 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. Of note, due to the underlying condition under study, ammonia values will often fall outside of the reference ranges; clinical significance and follow-up of these abnormal values will be determined by the treating Investigator on an individual subject basis.

An abnormal laboratory value that is not already associated with an AE is to be recorded as an AE if an action on IP 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.

## 9.7. Review of Safety Data

The designated pharmacovigilance physician in collaboration with the 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 in accordance with the medical monitoring plan. A Safety Review Committee comprising the Lead Investigator, Medical Monitor, pharmacovigilance physician, and an external disease expert, as needed, will meet at predetermined intervals during the study as well as in response to certain clinical outcomes. At each meeting, the Safety Review Committee will review blinded data from the ongoing study and determine whether the study should continue as planned, be modified, or be terminated. (Section 4.4). For details refer to the separate Safety Review Committee charter.

# 10. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

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 database lock and unblinding.

In addition to the analyses described within this section, interim analyses may be conducted.

## 10.1. Populations for Analysis

The following populations will be defined:

| | |
|---|---|
| **Safety Population (SAF):** | All subjects in Part 1 who received at least one dose of SYNB1020 and have at least one post-baseline safety assessment. All randomized subjects in Part 2 who received at least one dose of SYNB1020 or placebo and have at least one post-baseline safety assessment. Subjects will be included in the analysis according to the dose and IP received. |
| **Microbiotic-kinetic (MK) Population:** | Subjects in the SAF who completed the clinical study and were considered evaluable for analysis of the MK data. Subjects will be included in the analysis according to the dose and IP received. |
| **Pharmacodynamic (PD) Population:** | Subjects in the SAF who completed the clinical study and were considered evaluable for analysis of the PD data. Subjects will be included in the analysis according to the study part and IP received. |

Data will be excluded from the MK and/or PD population in case of any major protocol deviation that may influence the MK and/or PD outcome.

If not indicated otherwise, the analyses of safety endpoints will be conducted based on the SAF and all MK/PD analyses will be performed based on the MK/PD population.

## 10.2. Safety Variables

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®), and the severity of AEs and laboratory abnormalities will be graded using the NCI CTCAE, version 4.03. AEs will be tabulated by SOC and preferred term. Incidence tables of subjects with AEs will be presented for all AEs by maximum severity, SAEs, AEs assessed as related to SYNB1020/placebo, and AEs resulting in discontinuation of study dosing.

Clinical laboratory data, ECGs, and other safety assessments will be presented in by-subject listings and will be presented in tabular format, including absolute values and changes from baseline (if applicable), by study day.

## 10.3. Microbiotic-Kinetic Variables

The following variables will be calculated from the serial fecal sampling from each subject, if data support calculation. Further details on these calculations will be included in the SAP.

- $T_{lag}$ – the time of the first detectable fecal SYNB1020
- $T_{ss}$ – the time to steady-state
- $q_{max}$ – the maximum observed fecal SYNB1020 qPCR signal
- $T_{last}$ – the time of the last detectable fecal SYNB1020
- Half-life – the time for the fecal SYNB1020 qPCR signal to decline by 50%

A sample will be considered negative for the purposes of SYNB1020 clearance if it is below the limit of quantification or estimated to be below 0.1% of the administered dose.

The microbiotic-kinetic parameters of SYNB1020 will be summarized descriptively.

## 10.4. Pharmacodynamic Variables

Pharmacodynamic data will be presented in by-subject listings and will be presented in tabular and/or graphical format, including absolute values and changes from baseline, by study day.

## 10.5. Determination of Sample Size

Part 1: The sample size for the sentinel cohort (6 subjects) is primarily designed for empirical evaluation of safety and tolerability in subjects with cirrhosis.

Part 2: A sample of 24 subjects must complete all 3 ammonia AUC intervals (baseline, Day 5, and Day 6) to detect a 20% reduction in average daily ammonia ($AUC_{0-24}$/24, from a baseline of 70 µmol/L) with an approximate significance level of 10% and an approximate power of 90%. Significance and power are approximate based on the assumptions below. The number of subjects assumes that the standard deviation (SD) of spot ammonia is 36.4 µmol/L, half of the variance is between subject, and that the SD for average daily ammonia is as little as half that of spot ammonia. The spot ammonia SD is based on a weighted average of SD observed in a prior study of ammonia lowering in a similar population.[23] The variability of AUC may be 50% lower than spot ammonia based on the FDA summary basis of approval for glycerol phenylbutyrate for urea cycle disorders.[24]

Due to uncertainty in the assumptions of decrease in variability in AUC of ammonia relative to spot measurements in this population, up to 2 interim analyses for sample size re-estimation may occur after at least 12 subjects have completed all ammonia AUC assessments, and the total number of subjects in Part 2 of the study may be increased up to 40 at either point based on the SD of data observed. Additional subjects may be enrolled to ensure that at least the planned number of evaluable subjects complete the study.

## 10.6. Randomization and Blinding

Part 1: Open-label, all subjects will receive SYNB1020. Neither subjects nor investigators are blinded.

Part 2: Subjects will be randomized in a 1:1 ratio to receive either SYNB1020 or placebo. Randomization will be performed using a computer-generated central randomization scheme maintained by the Sponsor or designee. Both subjects and investigators will be blinded to study compound assignment, as will all other study personnel, with the exception of one unblinded pharmacist at the study site(s). In addition, an unblinded observer will be assigned to this study and will be independent of the study team. The unblinded observer will be able to review unblinded data as needed to aid in study progress, and this individual will be responsible for re-evaluation of the sample size as defined in Section 10.5. Details of unblinded roles will be provided in a separate Unblinding Guidance Document. Upon observation of a safety event that requires knowledge of IP assignment to determine event causality and further action and/or treatment (e.g., SUSAR), the Sponsor's Medical Monitor may be unblinded to IP assignment. A procedure for emergency access to a subject's treatment assignment within the interactive voice response system will be provided. The unblinding of any blinded team member will be documented.

## 10.7. Changes in the Conduct of the Study or Planned Analysis

Only the Sponsor may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between the Sponsor and the Investigator. The only exception is when the Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, the Investigator should inform the Sponsor and the full IRB within 1 working day after the emergency occurred. All amendments that have an impact on subject risk or the study objectives or require revision of the informed consent document must receive approval from the IRB prior to implementation.

# 11. DATA RECORDING, RETENTION AND MONITORING

## 11.1. Case Report Forms

Data will be collected using source documents through an electronic data capture (EDC) 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 days of a subject 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.

## 11.2. 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 IP. However, these documents should be retained for a longer period if required by the applicable legal requirements.

## 11.3. 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, fax, mail, email, or use of the EDC system, 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 to 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 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 subject 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 subjects during this study. However, because of the experimental nature of the IP, 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 subjects enrolled in the study.

## 11.4. 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.

# 12. REGULATORY, ETHICAL, AND LEGAL OBLIGATIONS

## 12.1. 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.

## 12.2. Institutional Review Board Approval

The Investigator must inform and obtain approval from the IRB for the conduct of the study at named sites, the protocol, informed consent documents, and any other written information that will be provided to the subjects and any advertisements that will be used. Written approval must be obtained prior to recruitment of subjects into the study and shipment of SYNB1020/placebo.

Proposed amendments to the protocol (see Section 10.7) and aforementioned documents must be submitted to the Sponsor for review and approval, then to the IRB. Amendments may be implemented only after a copy of the approval letter from the IRB has been transmitted to the Sponsor.

Per GCP guidelines, the Investigator will be responsible for ensuring that an annual update is provided to the IRB until the study is completed (i.e., finalization of the clinical study report) to facilitate continuing review of the study and that the IRB is informed about the end of the study. Copies of the update, subsequent approvals, and final letter must be sent to the Sponsor.

## 12.3. Regulatory Authority Approval

The study will be performed in accordance with the requirements of the US FDA and will also meet all of the requirements of ICH GCP guidance. Amendments to the protocol will be submitted to the FDA prior to implementation in accordance with applicable regulations.

## 12.4. Other Required Approvals

In addition to IRB and regulatory authority approval, all other required approvals (e.g., approval from the local research and development board or scientific committee) must be obtained prior to recruitment of subjects into the study and shipment of SYNB1020/placebo.

## 12.5. Informed Consent

Informed consent is a process that is initiated prior to the subject's agreeing to participate in the study and continues throughout the subject's study participation. It is the Investigator's responsibility (or designee) to obtain written informed consent from each subject after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any study procedures are initiated. Each subject should be given a copy of the informed consent document and associated materials. The original copy of the signed and dated informed consent document must be retained at the site and is subject to inspection by representatives of the Sponsor or regulatory authorities. If any amendments occur throughout the course of the study that affect the informed consent form (i.e., when new study procedures or assessments

have been added), all active subjects should be reconsented using the same process for the initial consent.

## 12.6. Patient Confidentiality

The Investigator must ensure that the subject's privacy is maintained. On eCRFs and other documents submitted to the Sponsor, including any required adverse event reporting, subjects will be identified by their assigned subject number. Documents that are not submitted to the Sponsor (e.g., signed informed consent documents) should be kept in a confidential file by the Principal Investigator.

The Investigator shall permit authorized representatives of the Sponsor, regulatory authorities, and Ethics Committees to review the portion of the subject's medical record that is directly related to the study. As part of the required content of informed consent documents, the subject must be informed that his/her records will be reviewed in this manner.

## 12.7. Disclosure of Information

Information concerning the study, protocol, processes, assessments, investigational product, scientific data, or other non-public information related to the study or the Sponsor and its representatives are confidential and remain the property of the Sponsor. The Principal Investigator may use this information for the purposes of the study only.

It is understood by the Principal 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 required to other clinical investigators and to regulatory authorities. In order to allow the use of the information derived from this clinical study, the Principal 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 results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

## 12.8. Publication of Study Data

The Sponsor encourages the scientific publication of data from clinical research studies. However, Investigators many not present or publish partial or complete study results individually without participation of the Sponsor. The Principal Investigator and the 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 coordination of study data publication and adequate review of data for publication against the validated study database for accuracy.

Qualification of authorship will follow the requirements of the International Committee of Medical Journal 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.

## 12.9. 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 subject 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 ethical principles that have their origin in the Declaration of Helsinki.

# 13. REFERENCES

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# APPENDIX 1. RANITIDINE PACKAGE INSERT

https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/018703s068,019675s035,020251s019lbl.pdf

# APPENDIX 2. WEST HAVEN GRADING SYSTEM

| Sign/Symptom | Grade |
|---|---|
| Trivial lack of awareness<br>Euphoria or anxiety<br>Shortened attention span<br>Impaired performance of addition | 1 |
| Lethargy or apathy<br>Minimal disorientation for time or place<br>Subtle personality change<br>Inappropriate behavior<br>Impaired performance of subtraction | 2 |
| Somnolence to semistupor, but responsive to verbal stimuli<br>Confusion<br>Gross disorientation | 3 |
| Coma (unresponsive to verbal or noxious stimuli) | 4 |

Source: Ferenci P, Lockwood A, Mullen K, et al. Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna 1998. Hepatology 2002; 35:716-21.

[redacted]

[redacted]

[redacted]

[redacted]

# APPENDIX 4. CHILD-TURCOTTE-PUGH SCORE TESTING

| Parameter | Assign 1 Point | Assign 2 Points | Assign 3 Points |
|---|---|---|---|
| Encephalopathy grade | None | Grade 1-2<br>(or precipitant-induced) | Grade 3 or 4<br>(or chronic) |
| Ascites | None | Mild/moderate<br>(diuretic-responsive) | Severe<br>(diuretic-refractory) |
| Serum bilirubin (mg/dL) | < 2 | 2 to 3 | > 3 |
| Serum albumin (g/dL) | > 3.5 | 2.8 to 3.5 | < 2.8 |
| PT (sec prolonged) or<br>INR | < 4<br>< 1.7 | 4 to 6<br>1.7 to 2.3 | > 6<br>> 2.3 |

Abbreviations: INR = international normalized ratio; PT = prothrombin time
Source: https://www.hepatitis.va.gov/pdf/Child-Pugh-score.pdf

Child-Turcotte-Pugh scoring is obtained by adding the score for each parameter:

Child-Turcotte-Pugh class:

- A = 5 to 6 points
- B = 7 to 9 points
- C = 10 to 15 points

# APPENDIX 5. MODEL FOR END-STAGE LIVER DISEASE (MELD)

MELD scores should be calculated using the following formula:

MELD = 3.78×ln[serum bilirubin (mg/dL)] + 11.2×ln[INR] + 9.57×ln[serum creatinine (mg/dL)] + 6.43

Any value less than 1 is given a value of 1 (i.e., if bilirubin is 0.8 a value of 1.0 is used).

Source: Kamath PS, Kim WR, Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). Hepatology 2007; 45 (3):797–805.