Study Title: VE303 for Treatment of Hepatic Encephalopathy (HE)

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A Randomized Controlled Trial of VE303 to Treat Hepatic Encephalopathy

VE303 for Treatment of HE

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

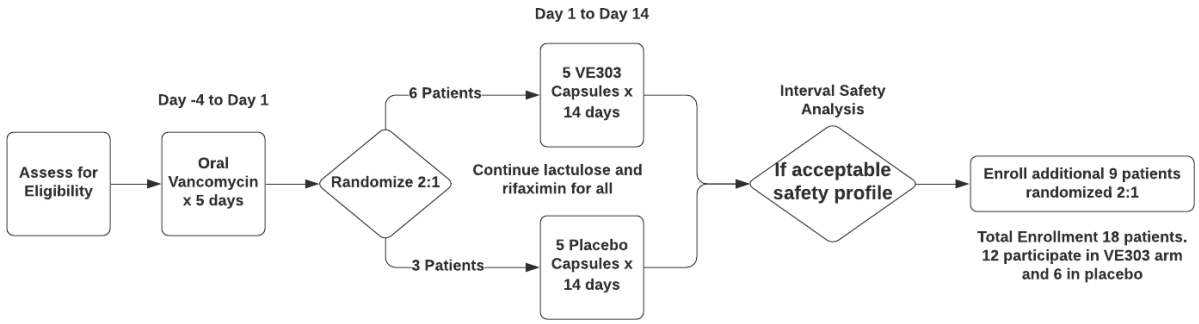
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A Randomized Controlled Trial of VE303 to Treat Hepatic Encephalopathy
Study Description:	This is a randomized controlled trial of VE303 in patients with a history of overt hepatic encephalopathy (OHE). VE303 is a live biotherapeutic product comprising 8 nonpathogenic commensal strains of Clostridia. The duration of intervention is approximately 18 days and post-intervention follow-up is 6 months. This is the first trial to use VE303 for treating this condition. Our hypothesis is that VE303 will reduce the number of recurrent OHE episodes. A detailed schematic describing all visits and a schedule of assessments are in the **Schema and Schedule of Activities, Sections 1.2 and 1.3,** respectively.
Objectives:	Primary Objectives: Evaluate the safety and tolerability of VE303 in patients with cirrhosis. Determine if VE303 improves cognitive function as assessed by psychometric HE score (PHES). Secondary Objectives: Determine if VE303 reduces hospitalizations for OHE in the 6 months after administration. Determine if VE303 changes the microbiome composition in cirrhosis, including colonization of VE303 strains. Determine if VE303 changes serum and stool biomarkers including short-chain fatty acids, bile acids, ammonia, and inflammatory markers.

Endpoints:	Primary Safety Endpoint: Number of serious adverse events up to Week 6 (4 weeks post VE303). Primary Efficacy Endpoint: Change in PHES as a measure of cognitive function from pre-vancomycin to Week 6 (4 weeks after VE303). Secondary Endpoints: - Number of hospitalizations for OHE up to Week 26. - Adverse events up to Week 26 - Change in patient reported outcomes from pre-vancomycin to Week 26 - Time to overt HE - Change in microbiome composition from pre-vancomycin to Week 26 - Change in serum and stool biomarkers from pre-vancomycin to Week 26 - PHES from pre-vancomycin to Week 26 - Stroop test at Week 4 and Week 26
Study Population:	The study sample will include 18 adult patients (aged 18-75 years old) with a history of cirrhosis and at least one prior episode of overt HE (OHE).
Phase:	Phase 2a trial
Description of Sites/Facilities Enrolling Participants:	This is a single-center blinded trial, enrolling only at the University of Michigan. Patients with cirrhosis and a history of OHE.
Description of Study Intervention:	All enrolled subjects will receive 5 days of oral vancomycin 125 mg QID. Starting the last day of oral vancomycin (Day 1), subjects will receive 5 capsules of VE303 or placebo daily for 14 days. VE303 and placebo capsules will be provided by Vedanta.
Study Duration:	18 months
Participant Duration:	7 months

1.2 SCHEMA



1.3 SCHEDULE OF ACTIVITIES (SOA)

	Pre-Screen	**Screening**	**Treatment Assessments** (O = Office, V = Virtual)				**Post-Treatment Assessments**			
	Day -40 to -4	Day -25 to -4 (0)	Visit 1, Day -4 (V)	Visit 2, Day 1 (O)	Visit 3, Day 8 ± 2 days (V)	Visit 4, Day 14 (O)	Visit 5, Week 4 ± 2 days (V)	Visit 6, Week 6 ± 2 days (O)	Visit 7-9, (Weeks 10, 14, 18 ± 2 weeks) (V)	Visit 10, week 26 ± 2 weeks (O)
Clinical Assessments										
Informed Consent		x								
Determine Eligibility	(in part)	x								
Randomize				x						
Demographics		x								
Medical History	x	x								
Symptom assessment, ER and hospital admissions		x	x	x	x	x	x	x	x	x
Full Physical Exam		x		x		x		x		x
Height		x								
Weight		x		x		x		x		x
Vital Signs[1]		x		x		x		x		x
Educational Session		x								
Medication List	x	x	x	x	x	x	x	x	x	x
Intervention adherence assessment[2]					x	x				
Lactulose/ Rifaximin Compliance	x	x	x	x	x	x	x	x	x	x
Speech assessment		x		x		x		x		x
PROMIS QOL test *10 min*		x		x		x		x		x
Bristol Stool Scale				x		x		x		x
PHES *15-20 min*		x		x		x		x		x
Stroop Test *10-20 min*		x		x		x		x		x
Collect solicited adverse events				x	x	x	x	x		
Collect unsolicited AEs			x	x*	x	x	x	x	x	x
Complete case report forms		x	x	x	x	x	x	x	x	x
Laboratory Assessments										
CBC + platelet		x		x		x		x		x
Basic metabolic panel		x		x		x		x		x
Liver panel		x		x		x		x		x
PT/INR		x		x		x		x		x
Amylase and lipase level				x		x		x		
Urine Pregnancy[3]		x								
Venous Ammonia		x		x		x		x		x
C.difficile test[4]		x								

Stool Sequencing Sample		x		x		x	x	x		x
Stool ammonia		x		x		x		x		x
Stool rifaximin level				x		x		x		
Stool vanc level				x						
Serum inflammatory biomarkers, LPS, SCFA		x		x		x		x		x
Stool bile acids		x		x		x		x		x
Stool SCFAs		x		x		x		x		x
Archive Stool		x		x		x		x		x
Archive Serum		x		x		x		x		x
Intervention										
Vancomycin			For 5 days							
VE303 or placebo				For 14 days	x	x				

[1] We will collect temperature, heart rate, and blood pressure
[2] For virtual visit, ask subject to show # of pills remaining in study pill bottle. For in person day 14 visit, count remaining pills in study pill bottle
[3] Women of childbearing potential
[4] *C.difficile* testing is with EIA test for CDI antigen and toxin A and B. If EIA test is inconclusive, the PCR will be run.
*For the time period between starting vancomycin and VE303 or placebo, subjects are instructed to contact the study team if they experience any adverse effects with taking vancomycin. Subjects will be asked about adverse events on day 1.
**If a patient's planned vancomycin start date is over 14 days after screening, an additional blood draw for a basic metabolic panel will be performed on Day -6 to check creatinine. Those with a serum creatinine 1.5 – 2.0 mg/dL at screening or on Day -6 will have an additional blood draw for basic metabolic panel obtained on Day -2 to check creatinine.

2 INTRODUCTION

2.1 STUDY RATIONALE

Hepatic encephalopathy (HE) is a complication of cirrhosis characterized by impaired cognition and abnormal psychomotor function. Up to 40% of patients with cirrhosis will develop HE, which leads to impaired quality of life, frequent hospital admissions, falls, and increased mortality.(1) First line HE treatment, lactulose, has limited efficacy with adverse effects that may include diarrhea, electrolyte disarray, dehydration, and patient discomfort.(2) Rifaximin, a second line treatment, is well tolerated but not accessible to many patients because of its costs and frequent insurance denial. The combination of lactulose and rifaximin is not completely effective at preventing recurrent HE episodes,(3) and both must be administered long-term after the onset of HE to prevent recurrence. More effective, less toxic and preferably finite therapies are needed to prevent overt HE episodes and treat minimal or subclinical HE that persists after overt HE (OHE) episodes. Manipulating the intestinal microbiome has been proposed to treat HE because of prior work demonstrating that HE develops through multiple mechanisms including from intestinal microbial products traversing the intestinal mucosa, crossing the blood-brain barrier, and affecting brain function. Indeed, lactulose and rifaximin as treatment of HE work in part through manipulation of the intestinal microbiome.

We are performing a randomized, placebo-controlled, double-blind trial of VE303 to prevent recurrent OHE and improve cognition in patients with cirrhosis and a history of OHE. VE303 is a live biotherapeutic product composed of 8 nonpathogenic, nontoxigenic strains of Clostridia developed to prevent C. difficile infection, with characteristics that make it a promising treatment for HE.

2.2 BACKGROUND

Currently available HE treatments are flawed. There are two available FDA-approved therapies: lactulose and rifaximin. Lactulose frequently leads to side effects, including diarrhea, bloating, electrolyte disarray,

and dehydration. Rifaximin, while causing fewer side effects, is costly and not covered by many insurance programs, thus limiting its accessibility to many patients. Finally, both these therapies are not perfect and many patients develop overt HE or experience ongoing mild HE symptoms despite treatment.

Manipulating the intestinal microbiome has been proposed to treat HE because of prior work demonstrating that HE develops at least in part from intestinal microbial products traversing the intestinal mucosa, crossing the blood-brain barrier, and affecting brain function.(4, 5) Microbiome-directed therapy could plausibly treat HE for several reasons: first, lactulose and rifaximin, current standard-of-care treatments for HE, improve cirrhotic microbiome dysbiosis and function.(6-8) Second, replacing the gut microbiome with bacteria lacking urease in a mouse model led to reduced ammonia production and improved neurobehavioral function.(9) Third, a meta-analysis found that probiotics given to patients with HE decreased plasma ammonia.(10) Fourth, two trials of fecal microbiota transplant (FMT) have demonstrated improved cognition in patients with a history of OHE.(11, 12)

Several plausible mechanisms link the intestinal microbiome to HE: 1) Intestinal dysbiosis in cirrhosis leads to **increased ammonia production** and therefore increased risk of HE. Evidence for this mechanism comes from cirrhotic patients with minimal HE who were found to have a significant linear correlation between intestinal concentrations of urease-containing *Streptococcus salivarius* and higher serum levels of ammonia.(13) Invasion of the distal intestine with oral commensals in cirrhosis perhaps explains the elevated abundance of these ammonia-producing Streptococcal species in the colons of these patients.(14) 2) **Elevated circulating pro-inflammatory cytokines observed in cirrhosis** increase the permeability of the blood-brain barrier to ammonia leading to poor cognition.(15-18) Investigators have found *Faecalibacterium prausnitzi*, a commensal with robust anti-inflammatory properties, is diminished in cirrhotic patients compared to healthy controls.(19-22) 3) Taxa with robust **short-chain fatty acid (SCFA)** production, essential to enterocyte health and maintenance of intestinal gap junctions, are diminished in cirrhotic patients, which may contribute to impaired intestinal permeability to neurotoxic bacterial products.(23) In a rat model, oral administration of SCFAs reduced enterotoxin-induced liver injury and TNF-α levels.(24) Based on this existing evidence, **we hypothesize that manipulation of the gut microbiome could improve cognitive deficits in cirrhotic patients by influencing the pathways linking the gut to HE and prevent recurrent OHE.**

VE303 is a promising treatment for HE. First, VE303 is designed to treat C. difficile infection (CDI), and HE has previously been successfully treated with another microbiome therapy for CDI – FMT. Second, VE303 contains organisms with robust SCFA production, one of the suspected pathways by which microbiome therapies could improve intestinal permeability and therefore HE. Third, VE303 strains induce conversion of primary bile acids into secondary bile acids, which has the potential downstream impact of reduced intestinal permeability. Fourth, VE303 strains induce T-regulatory cells, which play a critical role in maintaining intestinal homeostasis by controlling inflammation.(25, 26)

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Psychological Risk

Participation in research may lead to undesired changes in thought process or emotion. Patients may experience discomfort when being asked questions about their medical history that they deem to be private. Cognitive testing may create stress or frustration for patients. Finally, stool sample collection is psychologically troubling to some.

Risks of VE303

Thus far, VE303 has been given to approximately 100 people in clinical trials. The known or expected risks of the study medication are:

- Common (happens in 10 or more patients out of 100): diarrhea, nausea, and belly pain
- Uncommon (fewer than 10 patients out of 100): belly bloating, headache, elevated lipase (an enzyme from the pancreas), constipation, vomiting, and chills.

Most cases of abdominal distension and nausea were attributed to oral vancomycin pretreatment rather than VE303.

Please see the Investigator Brochure for further details regarding treatment emergent adverse events.

Risks of oral Vancomycin

From information obtained in the oral vancomycin capsule package insert, the most common adverse reactions (≥ 10%) were nausea (17%), abdominal pain (15%), and hypokalemia (13%). Nephrotoxicity has occurred following oral vancomycin capsule therapy, though not common, with those 65 years old and older at highest risk. This toxicity was reported in patients with colitis, which will be excluded in our cohort. We will monitor serum creatinine in all patients before and after vancomycin administration. Ototoxicity has also occurred in patients receiving oral vancomycin capsule, though not common. Finally, there is increased risk of the development of drug resistant bacteria.

Risks Related to Study Procedures

Subjects may develop a bruise, feel light-headed, faint or develop an infection related to study blood draws.

2.3.2 KNOWN POTENTIAL BENEFITS

This is the first study of VE303 in this patient population, so it is unclear if VE303 will provide any benefit. Potential benefits of VE303 include improvement in cognitive function and prevention of recurrence of OHE.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

HE is a common complication from cirrhosis, in desperate need of more effective and tolerable treatments. Discovery of one such novel treatment could benefit thousands of future patients. VE303 has demonstrated a good safety profile in healthy volunteers and individuals with CDI. VE303 contains only known commensal bacterial strains, and therefore poses no known risk for harm to patients with cirrhosis. HE is a condition associated with considerable risk itself; therefore, we believe that the potential for benefit with this trial outweighs the potential risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Primary Safety Objective is to determine if VE303 increases the rate of serious adverse events (SAEs).	Primary Safety Endpoint: Number of SAEs up to Week 6.	This is the first trial of VE303 in cirrhosis and safety assessment is a primary objective.

Primary Efficacy Objective is to determine if VE303	Primary Efficacy Endpoint: Change in	The neurological impairment associated with HE involves deficits in several domains:

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
improves cognitive function.	psychometric HE score (PHES) from pre-vancomycin to Week 6.	attention, visuospatial, fine motor skills and memory. The ideal tool is a validated assessment that comprehensively assesses these domains for impairment.(27) The Psychometric Hepatic Encephalopathy Score (PHES): • Battery of 5 paper-pencil tests that evaluate cognitive and psychomotor processing speed and visuomotor coordination. • 15-20 minutes to complete • Good external validity, endorsed by major specialty organization (ISHEN) and developed specifically to assess minimal HE. • Scores on each subtest are assigned values based on age-related norms
Secondary		
Secondary Objective 1: Determine if VE303 reduces OHE hospitalizations in the 6 months post-administration.	**Secondary Objective 1:** OHE hospitalizations in the 6 months post-administration (through Week 26).	Recurrent OHE is associated with considerable morbidity and mortality, and therefore has significant clinical importance.
Secondary Objective 2: Evaluate the durability of cognitive effect of VE303.	**Secondary Objective 2:** Change in PHES from pre-vancomycin to 6 months post-VE303 (through Week 26).	It is important to understand the durability of the intervention being studied.
Secondary Objective 3: Evaluate the safety and tolerability of VE303 by cirrhotic patients.	**Secondary Objective 3:** Difference between intervention and control arm in all adverse events	Safety is critical to evaluate with an investigational drug.
Secondary Objective 4: Determine if VE303 changes the microbiome composition in cirrhosis.	**Secondary Objective 4:** Change in microbiome composition. Calculate beta diversity between stool collection time	In order to understand the mechanisms underpinning the (potential) therapeutic effect of VE303 in HE, the microbiome changes associated with VE303 and improved cognition must be evaluated.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	points. We will perform metagenomic sequencing on stool to assess this. **Secondary Objective 5:** VE303 strain colonization, VE303 strain-specific marker sequences will be detected in the stool over time. We will perform metagenomic sequencing on stool to assess this.	
Secondary Objective 5: Determine if VE303 strains colonize cirrhotic patients.		The VE303 component strains have variable susceptibility to rifaximin. Monitoring VE303 strain colonization in this study population is critical to understanding the associations between VE303 and improved cognition.
Tertiary/Exploratory		
Determine if VE303 changes serum and stool biomarkers to explore the mechanism behind the gut-liver-brain connection.	Change over time in: 1. Venous and fecal ammonia 2. Inflammatory cytokines including IL-1, IL-6, IL-18, CRP, fecal calprotectin, TNF-alpha 3. Endotoxin (LPS) 4. Serum and fecal short-chain fatty acids 5. Fecal bile acids 6. Speech assessment	In order to better understand the mechanism underpinning the gut-liver-brain connection.

4 STUDY DESIGN

4.1 OVERALL DESIGN

We are performing a single-center randomized, placebo-controlled, double-blinded trial of VE303 to prevent OHE recurrence and improve cognition in patients with a history of overt hepatic encephalopathy (OHE). Our hypothesis is that VE303 will safely and effectively prevent OHE episodes and improve cognitive function, while changing the fecal microbiome in patients with a history of HE. Subjects will be

randomized 2:1 to VE303 vs placebo. There will be no stratification. Both study arms will receive 5 days of oral Vancomycin. The intervention arm will receive 5 VE303 capsules daily for 14 days and the placeboarm will receive 5 placebo capsules daily for 14 days. VE303 or placebo will start on the last day of Vancomycin (Day 1). Follow up will occur at Day 8, Day 14, and Weeks 4, 6, 10, 14, 18 and 26 after the start of VE303 or placebo. Depending on results from the initial pilot study, further study of different dose,duration, and the necessity of pre-VE303 Vancomycin may be required.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study will be the first investigation of safety and efficacy of VE303 in patients with cirrhosis. As such, we plan to perform this phase 2a study. Placebo capsules for VE303 exist, and will provide an excellent control for our experiment. Performing a randomized placebo-controlled study will provide a high level of evidence concerning the safety and efficacy of VE303.

In a study of healthy volunteers, pre-treatment with vancomycin was necessary for durable colonization of VE303 strains. In fact, VE303 strains were detected in recipients 1 year later when vancomycin pre-treatment was used. In order to optimize VE303 engraftment, VE303 must be administered as soon as possible after Vancomycin has been given. Delaying VE303 by even 1 or 2 days after Vancomycin completes can significantly reduce engraftment. For that reason, we plan to administer VE303 (or placebo) **on** the 5th day of Vancomycin; in other words, overlapping with Vancomycin for one day. If the patient unexpectedly requires rescheduling (for example due to a snow storm), we will allow for VE303 (or placebo) delivery on the day after Vancomycin completes, but will make strong efforts to dose VE303 (or placebo) exactly on Day 1 (last day of Vancomycin).

If only the VE303-treatment arm received pre-treatment Vancomycin, two substantial study design problems would emerge: 1) the experiment would no longer be blinded as both patients and investigators would be aware of their grouping, introducing potential observer bias; 2) any comparison of outcomes between the placebo and treatment arms would be confounded by the use of vancomycin in only the treatment group. It would be impossible to disentangle which outcomes were related to vancomycin and which were related to VE303. Finally, oral vancomycin has very few side effects or risks. As such, we plan to administer pre-treatment vancomycin to all participants.

All participants will remain on both lactulose and rifaximin at their prescribed dose and frequency throughout the study. First, maintaining both lactulose and rifaximin in all patients will prevent confounding or the need for stratification, which would have been necessary if there were heterogeneity in the active HE treatment of participants. Second, withdrawing lactulose and/or rifaximin prior to trial participation would risk precipitating an OHE event, which would pose too large a risk to participants. Third, rifaximin has weak anti-microbial properties and does not appear to impact microbial abundance in cirrhosis (rather impacts microbial function).(6)

4.3 JUSTIFICATION FOR DOSE

In a Phase 1 study of healthy volunteers, 14 days of 10 daily VE303 capsules achieved the greatest number of VE303 strains at 1 year of follow up. Since the completion of that study, Vedanta now has capsules that contain double the quantity of the original capsules. Therefore, only 5 capsules are now needed to yield the same dose. This dosing regimen had a tolerable and benign safety profile. Patients with cirrhosis are more likely than individuals with no cirrhosis to require multiple doses of FMT to treat refractory CDI.(28) This increased treatment requirement is likely due to the entrenched dysbiosis characteristic of cirrhosis.

As such, we suspect this 14-day dosing regimen is the most likely to overcome the dysbiosis of cirrhosis, safely, and lead to positive clinical outcomes.

In the placebo arm, we will administer placebo capsules on the same schedule as the VE303 arm, in order to maintain blinding.

Depending on results from the initial pilot study, further testing of different dose and duration, and need for vancomycin pre-treatment may be required.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3. The end of the study is defined as completion by the last participant of the last visit or procedure shown in the SoA section.

5 STUDY POPULATION

Eligible patients have cirrhosis, at least one prior episode of OHE any time in the past and no current OHE. Only patients consistently taking lactulose and rifaximin are enrolled to limit confounding. Additional exclusion criteria include ongoing use of systemic antibiotics or immunosuppression.

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Able to provide consent, with signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study, including ability to store VE303 or placebo in home fridge
3. Male or female, aged 18 through 75 years old
4. Diagnosis of cirrhosis based on liver biopsy, imaging, or evidence of clinical decompensation
5. History of at least one episode of overt HE any time in the past
   - Defined by West Haven Criteria Grades II to IV
   - Can be precipitated HE episode
6. Prescribed both lactulose and rifaximin, and compliant with this treatment
   a. Lactulose: At least one dose/day at least 5 days per week
   b. Rifaximin: At least one dose/day at least 5 days per week
7. Sexually active women of childbearing potential enrolled in the study must agree to use a highly effective method of contraception, other than oral contraceptive pills (defined in Appendix 1) until 3 months after the last dose of the study product.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current episode of overt HE as defined by West Haven Criteria Grades II to IV
2. Current infection including C difficile
3. Variceal bleeding in the last 4 weeks
4. Gut-absorbable or intravenous antibiotic therapy in the last 28 days
5. Anticipated antibiotics in the coming 4 weeks

6. Fecal microbiota transplant in the last 6 months
7. Use of probiotics in the last 2 weeks
8. Alcohol or illicit drug intake in the last 4 weeks
    - By history
    - Alcohol use will be characterized as >1 alcoholic drink / week
9. Primary sclerosing cholangitis as etiology of liver disease, as prior literature has suggested these individuals have a unique microbiome
10. History of inflammatory bowel disease, short gut, gastrointestinal tract fistulas, intestinal ischemia, or any form of ongoing colitis
11. Prior diagnosis of dementia or other primary neurocognitive disorder
12. Known hypersensitivity/allergy/intolerance to Vancomycin and any ingredients of VE303: sucrose, histidine, yeast extract, cysteine, metabisulfite, and microcrystalline cellulose
13. History of Roux-en-Y Gastric bypass
14. Any gastrointestinal surgery in the last year
15. Substantial immune compromise/deficiency (e.g., uncontrolled HIV, active immune suppressive therapy including high doses of corticosteroids or medications to prevent graft rejection, recent myeloablative therapy, sustained neutropenia)
16. Pregnancy or breast feeding
17. Use of oral contraceptive pills in the last month or planned use during the study period
18. MELD > 20
19. History of spontaneous bacterial peritonitis
20. Hemodialysis in the last 28 days
21. Other significant laboratory abnormalities:
    - Serum creatinine > 2.0 mg/dL
    - Hemoglobin < 8 g/dL
    - Serum sodium < 125 mmol/L
    - Serum potassium < 2.5 mmol/L
22. Placement of a portosystemic shunt or transjugular intrahepatic portosystemic shunt in the last 3 months (permissible if placed >3 months before enrollment)
23. Unstable doses of opiates, benzodiazepines or other sedating medication
    - Chronic methadone or low dose benzodiazepines (for example) is acceptable

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but do not receive any study drug, including vancomycin, VE303 or placebo. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, reasons for screen failure, and eligibility criteria.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a recent clinical event on the list of exclusion criteria may be rescreened. Rescreened participants should be

assigned the same participant number as for the initial screening. Subjects will be limited to one re-screen.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Subjects will be identified through multiple mechanisms. First, University of Michigan hepatologists will be asked to review their patient panel for potential candidates. Second, the schedule of patients presenting to University of Michigan Liver Clinic will be reviewed in advance to screen for potential eligible patients. If an eligible patient is identified, the hepatology provider will be notified. If the hepatology provider agrees that the patient is an appropriate potential subject, the subject will be approached after their clinic visit. Finally, a flier will be posted in the University of Michigan Liver Clinic. Potential subjects' hepatologist will be approached for permission to have study staff speak with the subjects.

Subjects will receive a $50 check for each in-person screening or study visit.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

VE303 is a living biotherapeutic product (LBP) consisting of 8 well characterized, clonally-derived, nonpathogenic, nontoxigenic strains of Clostridia. The composition of VE303 was rationally selected based on strain and consortia-specific properties for their association with healthy human microbiomes, lack of C. difficile-related dysbiosis, and lack of overt pathogenic features. Each of the 8 bacterial strains in VE303 produce SCFAs, which are reduced in patients with cirrhosis and may influence intestinal barrier function and the development of HE.

6.1.2 DOSING AND ADMINISTRATION

All enrolled participants will receive oral vancomycin 125 mg QID for 5 days prior to randomization. The purpose of this pretreatment is to reduce total bacterial biomass and allow for optimal VE303 strain colonization (see VE303 investigator brochure for further detail on rationale).

The patients randomized to VE303 will receive 14 days of 5 oral VE303 capsules, taken once daily. The patients randomized to placebo will receive 14 days of 5 oral placebo capsules, identical in appearance to VE303, taken once daily. The VE303 or placebo regimens will start on the last day of Vancomycin (Day 1), but could be given up to the day after Vancomycin stops if there are unavoidable schedule changes. Patients will be asked to take the study drug at approximately the same time each day.

VE303 and placebo capsules will be administered orally once daily with a beverage of choice that contains no solid food substances – water is ideal, but any clear or carbonated beverage may be used. Beverages should be at room temperature, cool, or warm (not hot).

In addition, patients cannot:

- Consume food or fast-acting antacids (calcium-, aluminum-, or magnesium-containing products such as Tums or Maalox) for at least 1 hour before or after study drug ingestion
- Ingest histamine-2 receptor antagonist (ranitidine, famotidine, etc) within 12 hours before or 1 hour after study drug ingestion
- Ingest proton-pump inhibitor (omeprazole, pantoprazole, etc) within the 12 hours before or 1 hour after study drug ingestion.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Study intervention and placebo product will be shipped from PCI Pharma Services (Rockford IL), to the University of Michigan Research Pharmacy. The University of Michigan Research Pharmacy will confirm receipt of the study products with Vedanta. The University of Michigan Pharmacy will store the study products in a 5°C +/- 3°C fridge. This fridge will be in the locked premises of the Research Pharmacy, and only accessible by Pharmacy staff. A log will be kept detailing receipt, dispensing, and destruction or return to Vedanta of study product, by Pharmacy staff and according to University of Michigan Research Pharmacy standard operating procedure.

Vancomycin will be supplied by the University of Michigan Research Pharmacy. Information on formulation, storage, and stability can be found in the package insert.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

VE303 consists of 8 different Clostridial strains, each manufactured separately. The quantity of each strain is proportioned to assure a specific per-strain per-capsule titer. The 8 strains are blended together with a micro-crystalline cellulose flow agent and placed in enteric capsules. The capsule also contains sucrose, histidine, yeast extract, cysteine, and sodium metabisulfite as excipients. The capsule will deliver $1.6x10^9$ CFU per strain per capsule. The capsules are designed to pass through the stomach and then release the strains in the upper small intestine. Further details are provided in the Investigator Brochure and Pharmacy Manual.

Placebo capsules contain micro-crystalline cellulose and are visually identical to VE303 capsules. However, placebo capsules will not contain any bacterial strains or formulation buffer used in the VE303 drug product.

6.2.3 PRODUCT STORAGE AND STABILITY

VE303 is stable when stored in the sealed and capped bottle at 5°C ± 3°C. The University of Michigan Research Pharmacy will store both the VE303 and placebo capsules in a fridge under these conditions until dispensed to the patient. When dispensed to the patient, the study drug will be contained in the capped bottle, in a lunch box with freezer packs. The patient will ingest the VE303 or placebo capsules on Day 1 under the observation of study staff and then will be instructed to transport the study drug directly to their home. They will keep the capsules in a contained box within their fridge, clearly labeled with the statement “Caution: New Drug — Limited by Federal (or United States) law to investigational use.”, and only for the subject’s use.

6.2.4 PREPARATION

The study product is produced to the point of capsule formation by the manufacturer. This study team at University of Michigan will not be involved in capsule creation. See the Pharmacy Manual for further detail regarding study drug preparation.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Subjects who have signed informed consent and meet enrollment criteria will be scheduled for a series of study visits. The first treatment study visit must take place within 3 weeks (or 21 days) of signing informed consent. If subjects are unable to present for the first treatment study visit within 21 days, we will re-verify eligibility including repeating lab testing and physical exam. Subjects will be randomized once they have signed informed consent and completed 4 days of oral vancomycin (5 days total of vancomycin but the 5th day overlaps with study drug initiation). Study staff will remain blinded to subject randomization until the final subject reaches the primary safety and efficacy endpoints (Visit 6). After this point, a portion of the study team will be unblinded to evaluate the primary outcome results. A portion of the study team, including the PI Dr. Bloom, will remain blinded until the end of the study. Only blinded study team members will be involved in evaluating adverse events, including with regards to possible relatedness to study drug.

Randomization will be performed by the University of Michigan pharmacy. The study blind may be broken if a subject has received study drug and developed a serious adverse event that was potentially related to study drug.

6.4 STUDY INTERVENTION COMPLIANCE

To ensure drug accountability, subjects will be asked to show study staff their study drug pill bottle (virtually) on Day 8 and (in person) on Day 14. On Day 14, the subjects will be asked to bring the bottle of study drug to demonstrate it is empty (i.e. all of the study drug had been consumed). Subjects will also be asked about study drug compliance on Days 8 and 14.

There will be an independent data safety monitoring board (DSMB) composed of a hepatologist, an infectious disease specialist and a statistician not involved in the study. That group will review AEs, SAEs, and enrollment.

Safety monitoring will be ongoing throughout the protocol. Dr. Bloom or a co-investigator will evaluate the subjects at the Day 1 visit, Day 14 visit, Week 6 visit, and final study visit (Week 26). The PI will review all study visit documents, including labs, within 2 business days of the tests results. AEs will be thoroughly assessed after each treatment visit by study investigators and documented in the source. The PI and study staff will send blinded aggregate reports on safety events to co-investigators and the DSMB on a monthly basis.

The PI will meet with study staff regularly to review study progress, subject status, and logistical issues. We will comply with the reporting of any IND safety reports according to FDA or other federal guidelines. The study coordinator will work with the physician investigators to process the report of these events as they happen.

Study monitoring will be performed by the University of Michigan Institute of Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. A site initiation visit will take place, followed by routine monitoring visits.

In addition to formal monitoring visits, Dr. Bloom will be regularly monitoring the study documents and meeting with study staff to review the status of the study. Dr. Bloom will ultimately be responsible for

protecting the rights, safety and welfare of all subjects enrolled in this study. In the case of Dr. Bloom's absence, monitoring responsibilities will be delegated to one of the study sub-investigators listed in the IRB application.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

To pass screening, subjects must report compliance with lactulose and rifaximin (at least one dose/day, 5 days per week for each of these medications), no gut-absorbable or intravenous antibiotics in the last 28 days, and antibiotic therapy cannot be anticipated in the coming 4 weeks. Subjects cannot be on immunosuppressive medications, or unstable doses of opiates, benzodiazepines or other sedating medications. These medications will be checked at each in-person study visit.

In addition, patients cannot:

- Consume food or fast-acting antacids (calcium-, aluminum-, or magnesium-containing products such as Tums or Maalox) for at least 1 hour before or after study drug ingestion
- Ingest histamine-2 receptor antagonist (ranitidine, famotidine, etc) within 12 hours before or 1 hour after study drug ingestion
- Ingest proton-pump inhibitor (omeprazole, pantoprazole, etc) within the 12 hours before or 1 hour after study drug ingestion.

6.5.1 RESCUE MEDICINE

The study will not supply rescue medication for overt HE. Need for rescue would be triggered by an episode of overt HE necessitating emergency room visit or hospital admission. The need for rescue medication will not be made by study staff, rather by treating providers. The use of rescue medication is allowable at any time during the study and will not be delayed for study purposes. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded.

If systemic antibiotics are deemed necessary during this study for a presumed infection, and VE303 component bacteria may be implicated, consideration should be given to the use of one or more of the 5 following broad-spectrum antibiotics, to which all of the bacteria contained in VE303 are susceptible (based on in vitro testing – refer to the VE303 Investigator Brochure for more details), according to institutional standard of care guidelines regarding antibiotic dose, route and frequency:

- Piperacillin/tazobactam
- Amoxicillin clavulanate
- Imipenem
- Metronidazole
- Tigecycline

This list of antibiotics will be reported to health care personnel if admission to the hospital or emergency department is warranted. Note: if VE303 component bacteria are implicated in an intestinal infection and an oral antibiotic is desired, oral metronidazole or amoxicillin clavulanate should be considered.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

We define temporary study-wide halting rules as follows:

a. Any serious adverse event (SAE) assessed as probably or definitely related to study treatment.
b. Any suspected or proven infection assessed as probably or definitely related to study treatment.
c. Two or more subjects with the same, or similar, Grade 3 or higher adverse event considered at least possibly related to the oral vancomycin and/or study drug (VE303 or placebo).

Temporary study-wide halt could entail:

a. Stop new enrollment but allow enrolled patients to continue treatment
b. Stop new enrollment and stop all dosing

In the event of an SAE, suspected or proven infection, or two or more subjects with the same or similar Grade 3 or higher AE, Dr. Bloom will assess the relatedness to study treatment. If Dr. Bloom assesses the event as probably or definitely related to study treatment, she will report the event to the data safety monitoring board (DSMB). The DSMB will review the case, make its determination about relatedness, and if deemed necessary, recommend a temporary study-wide halt. This recommendation may apply to (a) new enrollment only, or (b) both new enrollment and participants already being treated in the study. This event will be reported to Vedanta as well as the IRB according to the IRB reporting guidelines.

Study-wide halt will continue until relatedness to study treatment has been determined in consultation with (blinded) Vedanta personnel. Discontinuation from oral VE303 capsules does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol.

Once relatedness is determined by the DSMB and a decision has been made regarding a study halt, next steps, including plans to lift a study halt, would be discussed and agreed upon by Dr. Bloom, DSMB, in consultation with (blinded) Vedanta representatives, and the University of Michigan IRB. If the decision is made to discontinue participation in the study, discontinuation from oral VE303 capsules does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol.

The data to be collected at the time of study intervention discontinuation will include the following:

- Stool sample for culture and sequencing
- Routine blood tests, including CMP, CBC, INR
- Cognitive testing by PHES

In addition, an interval analysis will be performed when the 9th patient reaches Day 14 and has completed VE303 or placebo intervention. Specifically, the rate of SAEs, with a special focus on infection-related SAEs, will be compared between VE303 and placebo groups by the DSMB. If they deem the safety assessment to be satisfactory, the study will continue to complete enrollment of 18 subjects. We will briefly pause enrollment of the 10th patient, until this determination is made and restart enrollment immediately if there is no important safety concern related to VE303.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant. This includes, but is not limited to:
    - Single serious adverse event considered at least possibly related to the oral vancomycin and/or study drug (VE303 or placebo) treatment regimen
    - Grade 3 or higher adverse event considered at least possibly related to the oral vancomycin and/or study drug (VE303 or placebo) treatment regimen
    - Transmission of any infection from VE303
    - Pregnancy
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- If there is an interruption in dosing:
    - If participants miss more than one day of vancomycin dosing, the investigator will withdraw them from participation and allow them to re-screen and re-enroll in the future.
    - If one day of vancomycin is missed on Day -4, -3, or -2, then the participant will resume vancomycin and continue the study schedule.
    - If one day of vancomycin is missed on Day -1, the participant will be asked to take one extra day of vancomycin before randomization (so the next day is vancomycin only, and the day thereafter is vancomycin + study drug)
    - If the participant misses 1-2 days of VE303 or placebo, they will be instructed to continue the intervention and add those doses to the end of their treatment course
    - If the participant misses 3 or more days of VE303 or placebo, including for hospitalization:
        - If the interruption occurs on Days 12-14, continue the study as previously scheduled. Participants will not take the remaining capsules in this situation.
        - If the interruption occurs before Day 12 and they miss 3 or more days of study capsules, study capsules will not be resumed after the pause. They will continue all subsequent visits. Additional subjects may be enrolled to replace this subject.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Study Withdrawal Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but have not received the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and have received the study intervention, and subsequently withdraw from the study before the primary endpoint, not due to an SAE, may be replaced. Subjects who sign the informed consent form, and are randomized and have received the study intervention, and subsequently withdraw from the study due to an SAE, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks (1 week for study visits during treatment phase) and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The primary efficacy assessment is change in the Psychometric Hepatic Encephalopathy Score (PHES) from baseline (before vancomycin) to Week 6. PHES is a validated tool to measure hepatic encephalopathy disease activity.

The neurological impairment associated with HE involves deficits in several domains: attention, visuospatial, fine motor skills and memory. Therefore, we must use validated assessment tools that comprehensively assesses this impairment. The Psychometric Hepatic Encephalopathy Score (PHES) is the ideal tool to use. PHES is:

- Battery of 5 paper-pencil tests that evaluate cognitive and psychomotor processing speed and visuomotor coordination.
- Takes 15-20 minutes to complete.
- Good external validity, endorsed by major specialist organization (ISHEN) and developed specifically to assess minimal HE.(27)
- Scores on each subtest are assigned values based on age-related norms (1+ for scores better than 1 standard deviation (SD) above the normal mean to -3 for scores more than 3 SDs below the normal mean).
- Combined scores vary from +6 to -18.

The secondary efficacy assessment is number of OHE episodes up to Week 26 following study drug or placebo initiation. The presence of an OHE episode will be evaluated by the primary treating physician assessment during hospitalization, based on the West Haven Criteria.

Another secondary efficacy endpoint of this study will be change in PHES from pre-intervention assessment (screening visit) to Week 26.

As recommended by ISHEN, we will perform a second assessment of HE activity: the Stroop test. Stroop is also a well-validated test to screen and diagnose minimal HE.(29-31) The test involves identifying the color of the stimuli on the screen with three kinds of increasingly challenging stimuli. The test will be

performed on a study iPad, which will be maintained during the study period. The Stroop test sends an automatic email to study staff with results when the test has been completed. This email will be printed and saved as source documentation.

8.2 SAFETY AND OTHER ASSESSMENTS

The following assessments will be performed at screening and at several times during the study period to monitor safety (see SoA):

- **Physical examination** including neurological examination and volume assessment (for hepatic hydrothorax, LE edema, and ascites).
- **Vital signs** including body weight, temperature, pulse, and blood pressure. Study coordinator will be provided with a normal range of vital signs and will immediately notify a study physician of vital signs outside the normal range.
- **Laboratory evaluations**. A series of routine blood tests will be performed at screening and at 4 points throughout the study, as demonstrated in the SoA. These tests will include a BMP, LFTs, CBC, and INR. Amylase and lipase levels will be checked on Visits 2, 4, and 6. These test results will be sent to the PI's (Dr. Bloom) clinical inbox for immediate response. These blood tests will all be performed by the University of Michigan core lab facilities, in compliance with CLIA.
    - There is the potential for systemic absorption of oral vancomycin, which can be nephrotoxic at higher levels. Therefore, the following additional monitoring will be performed:
        - If a patient's screening labs were more than 14 days prior to planned vancomycin initiation, we will recheck serum creatinine on Day -6 (2 days prior to vancomycin initiation).
        - If at screening or on Day -6, the patient's serum creatinine was 1.5 mg/dL to 2.0 mg/dL (patients with serum creatinine > 2.0 mg/dL are excluded), these patients will undergo an additional blood draw on Day -2 to check serum creatinine. Vancomycin will be stopped if creatinine on Day -2 has increased to > 2.0 mg/dL or increased by 0.3 mg/dL since screening or Day -6. Vancomycin will not be resumed and the patient will not be randomized to study drug or placebo. The patient will continue to be monitored for nephrotoxicity but will be discontinued from the trial.
- **Assessment of adverse events.** Adverse event reporting will be documented by an adverse event reporting form on Days 1, 8, 14, Week 4, 6, 10, 14, 18 and 26 follow up visits. Adverse events will be graded based on Common Terminology Criteria for Adverse Events (CTCAE) V.5.0. If an adverse event is identified and is at least possibly related to VE303, a more intensive monitoring schedule may be employed. The default will be a daily phone call (during weekdays) from study staff until the adverse event has resolved or the participant is removed from the study. Depending on the severity of the adverse event, the PI may determine that phone calls every 2 or 3 days is appropriate. Conversely, the PI may determine that an in-office study visit is required for further evaluation.
    - We will actively collect the following solicited AEs from patients:
        - Diarrhea: This is challenging given that lactulose (a medication taken by all patients in the trial) purposefully creates loose and frequent stools. We will define diarrhea here as an increase in the number of bowel movements daily by 2 or more, or a noticeable increase in looseness of stool.

- Constipation: For this study, we will define constipation as no bowel movements for 2 days or more (if this is a change from baseline).
- Nausea
- Abdominal distension: This is challenging given that abdominal ascites and lactulose both cause abdominal distension in many of these patients at baseline. We will define this as a noticeable increase in abdominal distention from baseline or new abdominal distension.
- Abdominal pain
- Headache
- Chills
- Fever (temperature 100.4 or greater)

- We will give patients a thermometer and a diary to record solicited AEs. We will ask patients to record this list of solicited AEs daily in their diary from Day 1 through Day 21 (during 14 days of study drug and for 7 days thereafter). Study staff will specifically inquire about the solicited AEs during study visits 2, 3, 4, and 5 and collect the diary at visit 6.
- We will collect solicited and unsolicited AEs on separate forms. Solicited AEs will be kept in one large solicited AE case report form which will include all of a patients' solicited AE data from Day 1 through Day 21. Solicited AE data will be entered into this form on study visits 2, 3, 4, 5, and 6.
- Unsolicited AEs will be captured on a different case report form. Unsolicited AEs could occur during days 1-21 (while solicited AE recording is taking place) if they are a symptom not on the solicited list. If a symptom on the solicited AE list occurs after day 21 (when the patient is no longer being asked daily to record that symptom), that will be recorded as an unsolicited AE.
- In analysis, we will present the two types of AEs separately.

After the study screening visit, once all the screening assessments have been performed and resulted, a determination about screening failure or success will be made by the PI. The subject will be informed of the outcome and the remaining study visits will be scheduled if the subject was a screen success.

If any of the above safety assessments are abnormal, the participant will be informed of the results in a timely manner.

An interval analysis will be performed when the 9th patient reaches Day 14 and has completed VE303 or placebo intervention. Specifically, the rate of SAEs, with a special focus on infection-related SAEs, will be compared between VE303 and placebo groups by the DSMB. If the DSMB deems the safety assessment to be satisfactory, the study will continue to complete enrollment of 18 subjects. We will briefly pause enrollment of the 10th patient, until this determination is made and restart enrollment immediately if there is no important safety concern related to VE303.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. An emergency room visit which does not lead to hospitalization will not qualify as an SAE unless other criteria are met. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Grade 1: Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Grade 2: Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Grade 3: Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Of note, the term "severe" does not necessarily equate to "serious".
- **Grade 4: Life threatening**
- **Grade 5: Death**

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other

factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

The causal relationship between study drug administration and adverse events will be assessed based on several factors: First, the temporal relationship between drug administration and the adverse event. Adverse events that take place within 3 days of drug administration are more likely to be definitely, probably or possibly related. Second, in case of infectious adverse events, the isolated pathogen will clarify the likelihood of a causal relationship. VE303 contains exclusively Clostridial species. Third, the expert opinions of Drs. Bloom, Lok, and Young. Dr. Lok is an international expert in hepatitis and end stage liver disease, and will be able to significantly contribute opinions on whether the AE represents progression of underlying disease or the intervention. Dr. Young is an international expert on the microbiome and infectious disease and will be able to contribute an opinion regarding the likelihood that the AE is related to VE303. We appreciate this may be a challenging determination. As questions of relatedness arise in real time, a physician discussion between Dr. Bloom (sponsor investigator) and co-investigators Drs. Lok and Young will take place. Dr. Bloom will make the ultimate determination of relatedness, with input from Drs. Lok and Young. We will err on the side of caution in assessing events as related. The DSMB will ultimately review AEs and SAEs and can request further investigation into determinations and change the ultimate relatedness determination.

8.3.3.3 EXPECTEDNESS

Dr. Bloom will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention based on the protocol, informed consent, vancomycin package insert, and the VE303 investigator brochure.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed by PI), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

A clinical research coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until the last day of study participation (Week 26). At each study visit, the research coordinator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

All SAEs will be reported from a clinical research coordinator to Dr. Bloom, or Dr. Lok if Dr. Bloom is unavailable, within 24 hours. All non-serious AEs will be reported to Dr. Bloom within 5 business days.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

All SAEs will be reported from a clinical research coordinator to Dr. Bloom within 24 hours. Dr. Bloom will assess the relatedness to study treatment. If Dr. Bloom assesses the event as probably or definitely related to study treatment, she will report the event to the DSMB. The DSMB will review the case as outlined in the DSMB charter, make its determination about relatedness, and if deemed necessary, recommend a temporary study-wide halt. This event will be reported to Vedanta as well as the IRB.

The PI and study staff will send blinded aggregate reports on safety events including all SAEs to co-investigators and the DSMB on a monthly basis.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until Dr. Bloom deems the event to be chronic or the participant is stable.

All SAEs will be reported to the IRB per current institutional standards.

Dr. Bloom, with assistance from the University of Michigan Institute of Clinical and Health Research IND/IDE Investigator Assistance Program will be responsible for the reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32. This includes reporting of all Serious Adverse Events (SAEs) that are both unexpected and related to the drug as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. If the unexpected and related SAE is either fatal or life-threatening, then the SAE must be reported as soon as possible but in no case later than 7 calendars days after Dr. Bloom's initial receipt of the information. A summary of all non-expedited safety reports will be submitted in the annual report. In addition, Dr. Bloom must notify FDA and all participating investigators in this Investigational New Drug (IND) of any safety reports of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after Dr. Bloom determines that the information qualifies for reporting.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Participants will be informed of AEs and SAEs related to that participant as soon as they occur and will be updated as more information around relatedness becomes available. Any incidental findings, such as laboratory findings, will be disclosed to the participant, as well as their hepatology provider and primary care physician. Participants will not be informed of their cognitive testing results; only insofar as whether or not their score qualifies them to successfully screen into the study.

8.3.8 EVENTS OF SPECIAL INTEREST

NA

8.3.9 REPORTING OF PREGNANCY

If a woman becomes pregnant while in the study, the study drug will be discontinued and the team will request permission to follow the pregnant woman to her pregnancy outcome. The PI will notify the IRB and Vedanta within 24 hours.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) per current institution guidelines. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the sponsor investigator within 2 days of the investigator becoming aware of the event.

- Any other UP will be reported to the IRB and to the study sponsor within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 14 days of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be informed of unanticipated problems as soon as they occur, and will be updated as more information around relatedness becomes available.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Safety Endpoint: Number of SAEs from initiation of vancomycin up to Week 6.
    - Hypothesis: The intervention arm will not have a higher rate of serious adverse events.

- Primary Efficacy Endpoint: Change in psychometric HE score (PHES) from pre-vancomycin to Week 6.
    - **Null hypothesis**: The change in psychometric HE score (PHES) from pre-vancomycin to 4 weeks post-treatment (Week 6) is equal in the VE303/study drug and placebo/control arms.
    - **Alternative hypothesis**: The change in psychometric HE score (PHES) from pre-vancomycin to 4 weeks post-treatment (Week 6) will be greater (and beneficial) in the VE303/study drug arm than the placebo/control arm.

- Secondary Efficacy Endpoint 1: number of OHE hospitalizations in the 6 months following study drug administration (up to Week 26)
    - **Null hypothesis**: Patients who undergo the study intervention (VE303) will have the same number episodes of OHE in the 6 months following drug administration compared to those who received placebo.
    - **Alternative hypothesis**: Patients who undergo the study intervention (VE303) will have fewer episodes of OHE in the 6 months following drug administration than those who received placebo.

- Secondary Efficacy Endpoint 2: change in psychometric HE score (PHES) from pre-intervention to end of study follow-up (Week 26)
    - **Null hypothesis**: The change in psychometric HE score (PHES) from pre-intervention to the end of study follow up is equal in the VE303/study drug and placebo/control arms.
    - **Alternative hypothesis**: The change in psychometric HE score (PHES) from pre-intervention to the end of study follow up will be greater (and beneficial) in the VE303/study drug arm than the placebo/control arm.

- Secondary Efficacy Endpoint 3: Diversity from pre-intervention to post-intervention (Week 6)

- Hypothesis: The administration of VE303 will be associated with a greater diversity in the microbiota (as assessed by metagenomics) between the two time points as compared to placebo.

9.2 SAMPLE SIZE DETERMINATION

Given the short duration of follow-up (6 weeks) until the primary safety outcome, we predict <10% patient drop out.

This is a small pilot phase 2a study focused on safety as the primary outcome. Given 2:1 randomization, 12 patients will receive VE303. This is similar to other safety studies in this population.(11, 12)

9.3 POPULATIONS FOR ANALYSES

We will analyze safety and efficacy data according to a modified Intention-to-Treat Analysis (i.e., all subjects who were randomized and received at least one dose of VE303 or placebo).

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

In this small pilot phase 2a study, the focus will be on evaluating safety. After enrollment of the first 9 patients, we will perform interim analysis to evaluate safety of VE303 in this population. If there is no concern for a higher rate of SAEs in the VE303 group, enrollment will continue to reach a total of 18 patients. Results from all 18 patients will be used together to evaluate safety and efficacy at trial completion.

Demographic characteristics that are continuous variables with a normal distribution will be reported as a mean ± standard deviation. Continuous variables with a skewed distribution will be reported as median and interquartile range. Normality will be determined by the Shapiro-Wilk test. Demographic characteristics that are binary or categorical variables will be reported as a proportion in each category. If the continuous outcome is normally distributed, then we will perform a t-test and report the mean ± standard deviation as well as the confidence intervals. If the outcome is not normally distributed as determined by the Shapiro-Wilk test, we will perform a Wilcoxon Rank Sum test and report the median and interquartile range.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

If the continuous primary outcome is normally distributed, then we will perform a t-test and report the mean ± standard deviation as well as the confidence intervals. If the primary outcome is not normally distributed as determined by the Shapiro-Wilk test, we will perform a Wilcoxon Rank Sum test and report the median and interquartile range.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

If the continuous secondary endpoint is normally distributed, then we will perform a t-test and report the mean ± standard deviation as well as the confidence intervals. If the secondary endpoint is not normally distributed as determined by the Shapiro-Wilk test, we will perform a Wilcoxon Rank Sum test and report the median and interquartile range. If the secondary endpoint is categorical, we will perform a Fisher's exact test.

9.4.4 SAFETY ANALYSES

We will compare the rate of serious adverse events per patient between the intervention and control arms.

Laboratory data, including MELD score, if normally distributed will be tested by t-test and reported as mean ± standard deviation. If not normal, we will perform a Wilcoxon Rank Sum test and report the median and interquartile range.

In this small population, we will not compare the overall incidence of adverse events between groups, but will report these descriptively. We will report the solicited and unsolicited AEs separately.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Demographic characteristics that are continuous variables with a normal distribution will be reported as a mean ± standard deviation. Continuous variables with a skewed distribution will be reported as median and interquartile range. Normality will be determined by the Shapiro-Wilk test. Demographic characteristics that are binary or categorical variables will be reported as a proportion in each category.

9.4.6 PLANNED INTERIM ANALYSES

After the 9th patient reaches Day 14 of the study, an interim analysis will be completed. Safety events will be evaluated at the interim analysis. If there are no concerning findings in the safety outcome, the study will proceed to a total enrollment of 18 subjects. The interim analysis will be performed by the DSMB of1 hepatologist, 1 infectious disease physician and 1 statistician.

9.4.7 SUB-GROUP ANALYSES

The primary analysis will be stratified by the presence of TIPS. No other sub-group analyses are planned.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant PHES scores, Stroop test scores, ammonia levels, and microbiome data will be listed by time point.

9.4.9 EXPLORATORY ANALYSES

Computational analyses will assess VE303 strain penetration in the recipient microbiome by comparing single-nucleotide variants in strain level data between the donor and recipient, with the primary comparison being post-vancomycin/pre-VE303 (Day 1) compared to the final day of VE303 (Day 14). We will also calculate beta diversity between time points to assess change in microbial diversity before and after vancomycin, and over post-VE303 follow-up. We will examine taxa that differ significantly between pre- and post-VE303 administration, and test the degree to which changes in cognitive outcomes are explained by changes in the abundance of species and functional pathways.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A study physician investigator will obtain informed consent. In the case that a patient is in the medical practice of one of the investigators, the patient will have their information session and informed consent performed by another investigator. The consent form and protocol will be reviewed with the potential subject and any questions will be answered. Subjects will have the opportunity to take the consent form home and have 2 weeks to decide if they wish to participate.

Ability to provide informed consent will be determined by study investigator assessment of capacity and a discussion with the subject's primary hepatologist and, if needed, a mini-mental status exam. This study is designed to investigate a novel therapy for patients with a history of hepatic encephalopathy. Many patients with a history of hepatic encephalopathy have persistent cognitive deficits between overt episodes of encephalopathy. If ability to consent is in question, a mini-mental status exam will be performed. Subjects will require a score of 18 or greater in order to provide their own consent. A study physician investigator will review the consent form with each subject and perform a capacity assessment. Subjects will demonstrate capacity by being able to: 1) express an understanding of VE303 and its risks and benefits, 2) express a choice about study participation, 3) describe how the study may have an impact on his or her life, 4) express a comparison between choosing to participate and not.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Sponsor Investigator (SI) will promptly inform study participants, the

Institutional Review Board (IRB), sponsor, and FDA and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor investigator, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored the University of Michigan during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor investigator requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on the University of Michigan server. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, the data linking study identification number to participants identifying information will be destroyed. The deidentified database will be archived on the University of Michigan server.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the University of Michigan Gastroenterology and Hepatology Division and at Vedanta Biosciences, Inc., and may be used academically by the

University of Michigan Gastroenterology and Hepatology Division and commercially by Vedanta Biosciences, Inc.

With the participant's approval, de-identified biological samples will be stored in the University of Michigan Gastroenterology and Hepatology Division and at Vedanta Biosciences, Inc. These samples could be used academically and commercially for purposes including, but not limited to, researching the causes of liver disease, hepatic encephalopathy, and other conditions for which individuals with chronic liver disease are at increased risk, and to improve treatment.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed or after the samples have already been analyzed.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Sponsor Investigator
Patricia Bloom MD
University of Michigan
Taubman Center, 1500 E. Medical Center
978-460-0538
ppbloom@umich.med.edu

10.1.6 SAFETY OVERSIGHT

An independent data safety monitoring board (DSMB) comprised of an independent hepatologist, infectious disease physician, and a statistician will monitor the study.

In addition to formal monitoring visits by University of Michigan Institute of Clinical and Health Research (see 10.1.7), Dr. Bloom will be regularly monitoring the study documents and meeting with study staff to review the status of the study. Dr. Bloom will ultimately be responsible for protecting the rights, safety and welfare of all subjects enrolled in this study. In the case of Dr. Bloom's absence, monitoring responsibilities will be delegated to one of the study sub-investigators listed in the IRB.

10.1.7 CLINICAL MONITORING

To assure adequate protection of the rights of human subjects per 21 CFR part 312, this study will be monitored by the University of Michigan Institute of Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. A site initiation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Sponsor-Investigator.

The established monitoring plan will ensure the quality and integrity of the data throughout the study conduct to verify adherence to the protocol, completeness and accuracy of study data and samples collected, dispensing and inventory of the study drug, and compliance with regulations. Monitoring

visits can be conducted onsite or remotely with appropriate electronic documentation provided to the study monitors for review.

Blinded aggregate data on safety events will be sent from the PI and study staff to co-investigators and the DSMB.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

We will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the PI, Dr. Bloom, for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into RedCap, a 21 CFR Part 11-capable data capture system provided by the University of Michigan. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents will be retained until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor investigator, if applicable.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or International Conference on Harmonisation Good Clinical Practice (ICH GCP). The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 3 working days of the scheduled protocol-required activity. Protocol deviations must be sent to the Institutional Review Board (IRB) per their policies.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 2 years after the completion of the study by contacting Dr. Patricia Bloom.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 ADDITIONAL CONSIDERATIONS

NA

10.3 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HE	Hepatic encephalopathy
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
OHE	Overt hepatic encephalopathy
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event

SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
V2	3/15/2021	Addition of amylase and lipase monitoring. Enhanced creatinine monitoring with vancomycin. Addition of solicited AEs. Added detail to individual and study-wide halting rules.	Response to FDA request.
V3	3/24/2021	Addition of the following exclusion criteria: any GI surgery in the last year Revision of protocol number and addition of IND number	Response to FDA request. Minor editorial changes
V4	5/16/2021	Add exclusion criteria for oral contraceptive pills	Response to IRB Minor editorial changes
V4	6/10/2021	Add NCT number	Response to clinicaltrial.gov team request.
V5	4/1/2022	Fix discrepancy on page 16 and 27 of the protocol on when the interim analysis would occur. After the first 9 patients have completed treatment, an interim analysis will be completed.	Investigator initiated edits. Discrepancy identified by PI.
V6	11/28/2022	Updated for recent VE303 IB update and also to plan for unblinding for end of study analyses	New VE303 IB. Planning for end of study analyses.

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12 APPENDIX 1

CONTRACEPTIVES[a] ALLOWED DURING THE STUDY INCLUDE:
Highly Effective Methods[b] That Have Low User Dependency *Failure rate of < 1% per year when used consistently and correctly.*
• Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
• Intrauterine device (IUD)
• Intrauterine hormone-releasing system (IUS)[c]
• Bilateral tubal occlusion
• Azoospermic partner (vasectomized or due to a medical cause) *Azoospermia is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.* Note: documentation of azoospermia for a male participant can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
Highly Effective Methods[b] That Are User Dependent *Failure rate of <1% per year when used consistently and correctly.*
Sexual abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*
a) Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies. b) Failure rate of < 1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly. c) Male condoms must be used in addition to hormonal contraception. If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action. Note: Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM) are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure from friction).

Table obtained from the TransCelerate Protocol Library