# INTERVENTIONAL RESEARCH PROTOCOL

**STUDY INFORMATION**

- **Title of Project:**
  Modulation of gut microbiota with NBT-NM108 as an early treatment for suspected or confirmed symptomatic COVID-19 patients(COVGUT20)

- **Principal Investigator Name**
  Asa Oxner, MD FACP
  Liping Zhao, PhD

- **Principal Investigator Div. & Dept.**
  Dr. Asa Oxner:
  Department of Internal Medicine,
  University of South Florida Morsani College of Medicine

  Dr. Liping Zhao:
  Department of Biochemistry and Microbiology
  School of Environmental and Biological Sciences
  Rutgers University

- **Principal Investigator Contact Info:**
  Dr. Asa Oxner:
  [redacted]

  Dr. Liping Zhao:
  [redacted]

- **Protocol Version Date:**
  April 28, 2022

- **NCT:** NCT04540406

**Table of Contents**

Skip To Section: Hold **CTRL** + **Click (Below)** To Follow Link in **Blue**

## 1.0 Research Design

### 1.1 Purpose/Specific Aims

To determine feasibility and explore the relevance of modulating the gut microbiota by an Investigational New Drug NBT-NM108 as an early treatment for suspected or confirmed symptomatic COVID-19 patients.

#### A. Objectives

To determine the safety, feasibility, and effectiveness of using NBT-NM108 to modulate the gut microbiota in patients who have suspected or confirmed COVID-19

#### B. Hypotheses / Research Question(s)

Overgrowing opportunistic pathogens in gut microbiome may be one of the major driver for developing more severe complications in patients who have COVID-19. Our overall hypothesis is that supplementing COVID-19 patients with a combination of fermentable dietary fibers of diverse physicochemical structures provides an additional energy source that will promote a select group of acetate and butyrate producers in the gut. Akin to the tall trees as the foundation species for a closed forest, this group of bacteria may work as the "Foundation Guild" in the gut microbiota that change the gut environment and suppress the overgrowing opportunistic pathogens, which may reduce the severity of COVID-19-related illness.

### 1.2 Research Significance

The world is facing the COVID-19 pandemic induced by the virus SARS-CoV-2 [1]. Some COVID-19 patients with pre-existing conditions have a significantly higher risk of developing a severe form of the disease, higher hospitalization rate and increased mortality compared with the average patient population [2, 3]. Immunopathology induced by cytokine storm and sepsis from opportunistic pathogens are among the most common causes of mortality in COVID-19 patients, particularly those with those with pre-existing conditions [4].

A dysbiotic gut microbiota may be a major driver of the more severe clinical outcome in COVID-19 patients[5]. The dysbiotic gut microbiota has significantly higher abundance of opportunistic pathogens and toxin producers, including endotoxin-producing pathogens and producers of hydrogen sulfide [6]. These opportunistic pathogens increase the antigen load and sustain a higher level of inflammation [7]. If gut barrier function is impaired, these pathogens may translocate to internal organs and induce sepsis [8]. Viral infection to the intestinal epithelial cells can impair the gut barrier and increase gut permeability [9]. Thus, patients with dysbiotic gut microbiome may have a higher risk of developing a more severe form of the infection with catastrophic clinical outcomes due to: 1) a higher level of baseline inflammation with a greater risk of developing immunopathology; 2) more abundant opportunistic pathogens in the gut with a higher risk of developing sepsis; and 3) more severe gut barrier impairment if viral gut infection and hyperglycemia co-occur.

This randomized controlled trial will use a high fermentable dietary fiber formula (NBT-NM108, Notitia Biotechnologies, Wilmington, DE) as perturbation to improve the gut microbial ecosystem of patients who have COVID-19-like symptoms. We hypothesize that the predominance of a select group of acetate and butyrate producers supported by the additional fiber as an energy source may work as the "Foundation Guild" in the gut microbiota that can suppress the overgrowing opportunistic pathogens. Akin to the tall trees of a forest, members of the Foundation Guild maintain a healthier gut microbiota structure by producing short-chain fatty acids (SCFAs), particularly acetate and butyrate, that can make the gut environment inhibitory to opportunistic pathogens by: 1) acidifying the gut; 2) producing antimicrobials; and 3) taking up more available niches. Our recent clinical trial showed that a high-fiber diet effectively promoted the Foundation Guild in T2DM patients and shifted the gut microbiota structure that led to better clinical outcomes [6]. Studies in mice and chickens with various respiratory virus infections showed that acetate and butyrate from fermentable fibers significantly reduced disease severity and boosted antiviral immunity [10-15].

### 1.3 Research Design and Methods

#### A. Research Procedures

**RECRUITMENT.** We are recruiting patients who have mild to moderate COVID-19-like symptoms and pending/positive SARS-CoV-2 PCR test results. Prospective participants will be identified using two strategies: 1) online advertisements via Google, Facebook and Twitter will be sent to residents of mainland US. A link to the landing page of the study will also be distributed to family physicians and doctors in the USF and Rutgers University medical schools and facilities, and patient organizations via email, text and messaging apps for them to share with their patients and networks. Webinar will also be hosted to general public and information about this clinical trial will be posted. 2) individuals who have been tested positive for COVID-19 using services from Vault Health company, at the Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center at University of South Florida (USF) or at Rutgers University medical schools. An email (Appendix 25) will be sent to potential participant with a link to the landing page of the study.

**ELIGIBILITY SCREENING PART 1.** *For online recruitment*, individuals who are interested in participating in the study will access the landing page of the study (Appendix 19) via the online advertisements (Appendix 20) or a link shared by their family physicians, Vault Health, USF or Rutgers University. The landing page will provide an overview of the study and a brief description of requirements for research participants. Study candidates will provide their email addresses to access an online self-administered preliminary screening survey (Appendix 22). At the completion of the survey the study candidates will be informed whether they may be eligible for the study, and if so they will be instructed to schedule a virtual informed consent interview with the research team using 10To8 or phone calls. For female study candidates who are of child-bearing age (16-49 years), they will receive a pregnancy test kit (Rapid Detection Pregnancy Test, Clearblue, Geneva, Switzerland), perform the test at home and report their pregnancy status to the research team on Castor (Appendix 6) during the informed consent interview. *For recruitment from Vault Health company, at the Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center at University of South Florida (USF) or at Rutgers University medical schools*, individuals who has COVID-19 related symptoms and has been tested positive for COVID-19 will be contacted by Vault Health or research staff in USF/Rutgers University. An email/phone call containing a brief description of the study and a link to the landing page will be sent to the potential participants. If the study candidate is interested in the study, they will provide their email addresses on the landing page. And the following procedure will be the same as described in section for online recruitment.

**INFORMED CONSENT AND ELIGIBILITY SCREENING PART 2.** The informed consent process will be conducted via Castor's eConsent video call module. At the beginning of the call, the study coordinator will confirm that the study candidates continue to experience the COVID-19-like symptoms reported in the preliminary screening survey, thereafter the study coordinator will explain the rationale, aim, expected outcomes and significance of the research, the experimental procedures, what is required from the participants, and the benefits and risks associated with the study. Electronic informed consent will be obtained during the interview prior to further eligibility screening (Appendices 3 and 4). A consent for health information release (Appendix 3) will also be obtained during informed consent procedure. After the study candidates sign the electronic informed consent documents, the study coordinator will ask some additional questions regarding their medical history and other conditions to establish eligibility (Appendix 4). In the case of study candidates who are recruited online, they will also be asked to provide electronic proofs of a positive/pending COVID-19 test (Appendix 24) and a negative pregnancy test result (if appropriate; Appendix 6) during the interview. This consent and eligibility screening interview will take a maximum of 60 minutes to complete.

**ENROLLMENT.** The study physicians will review all screening data and determine if the study candidates are suitable for home isolation and confirm enrollment.

**RANDOMIZATION AND ALLOCATION CONCEALMENT.** Enrolled participants will be randomized to the control or treatment group at a 1:1 ratio, using randomization via a dynamic minimization to minimize imbalance between groups over factors including COVID-19-like illness severity (mild vs moderate), the use of COVID-19 vaccination or other treatments that may reduce severity such as monoclonal antibody treatment vs. not used, age (≤60 vs >60 years) and gender (male vs female) [16]. When imbalance is detected, the new patient will be allocated with probability 0.7 vs 0.3. When the treatment assignments are at balance, the patient will be allocated with probability 0.5 vs 0.5. The randomization will be performed

via a closed source app written by one of the Co-PI. The investigator who uses the app will only know the allocation result, i.e., control or treatment group, of the new patient.

**INTERVENTIONS.** All enrolled participants will receive self-supportive care for the treatment of COVID-19 and they will have access to a series of videos specific to their assigned group that provide them with an overview of the study schedule and what is required of them during the study (Appendix 16). The research team will provide participants with a digital oral thermometer (Medtus, MO) to monitor body temperature (oral), and a pulse oximeter (IMDK, Shenzhen, China) to monitor oxygen saturation level and pulse rate, and a glucometer (Curo Fit, Yorba Linda, CA) to monitor blood glucose if patients have type 2 diabetes mellitus (T2DM), with step-by-step instructions (Appendices 8.1, 8.2 and 16). The treatment group will take NBT-NM108, an Investigational New Drug from Notitia Biotechnologies, that provides fermentable dietary fibers as perturbation to improve the gut microbiota. NBT-NM108 will be taken in the form of drinks [redacted] (before each main meal and 2 h after dinner) from Day 1 to Day 28. Each drink will be prepared by mixing a sachet of NBT-NM108 [redacted]. The control group will follow the same schedule except they will drink the same volume of water without NBT-NM108. After 28 days of intervention, all participants will remain in the study for follow-up until Day 56. If participants require hospitalization during the study, they will discontinue all study procedures during hospitalization but remain in the study for follow-up until Day 56. Participants will have access to an interactive online personal dashboard to review their study progress and the data that they have reported (Appendix 17).

All of the devices and instructions required for the patient's participation will be shipped to the patient's home address via FedEx overnight shipping upon enrollment and randomization.

**SAMPLE AND DATA COLLECTION.** All participants will collect fecal samples at Days 0, 28, 56, and the day when they recover from COVID-19-like illness (if before Day 56). Participants are considered having recovered from COVID-19-like illness when they fulfil the CDC guidelines for discontinuing home isolation for persons with COVID-19 who have symptoms (updated July 20, 2020): at least 10 days have passed since symptom onset, at least 24 h have passed since resolution of fever without the use of fever-reducing medications, and other symptoms have improved. Participants will be provided with supplies and instructions (Appendices 8.1, 8.2 and 16) to collect fecal samples at home using a collection kit developed by [redacted] describe the fecal sample on a data collection form based on the Bristol Stool Scale (Appendix 12) [17] and mail the samples and the form to the research team in a prepaid shipping box by following the written instructions in Appendix 18. For participants who require hospitalization during the study, they will collect a fecal sample after they recover (on or before Day 56) and repeat sample collection on Days 28 and 56 as appropriate.

All participants will evaluate objective and subjective symptoms, adverse events and medication changes twice a day (in the morning around 8 am and 2 h after dinner around 8 pm using the last 12 h as the recall period) and record fasting blood glucose if participant has T2DM (after at least 8 h of overnight fasting) and consumption of NBT-NM108 (treatment group) or water (control group) once a day from Day 1 to Day 28 or until they recover from COVID-19-like illness (whichever is later) and then once a week until Day 56 (i.e. in the mornings of Days 35, 42, 49 and 56). Participants will also report symptoms, fasting blood glucose if participant has T2DM and medication changes on the day that they recover from COVID-19-like illness (if before Day 56). For measurement of body temperature (oral), oxygen saturation level, pulse rate and respiratory rate and fasting blood glucose if participant has T2DM, participants will be provided with devices and instructions to take the measurements; for the remaining subjective symptoms, participants will be asked to evaluate the severity of each symptom using categorical options of "none", "mild", "moderate", and "severe" or the severity of the symptoms as directed. Participants will be asked to record these data and any adverse events in the participant booklet (Appendices 8.1 and 8.2) and report to the research team on Castor (Appendices 9-11). For participants who are hospitalized during the study, they will stop collecting data themselves during hospitalization but body temperature (oral), pulse rate, respiratory rate, oxygen saturation level, fasting blood glucose if participant has T2DM, respiratory support and other data related to the clinical progress of COVID-19-like illness will be extracted from their medical records at the hospital. If these patients are discharged on or before Day 56, they will collect data on objective and subjective symptoms and medication changes twice a day and fasting blood glucose if

participant has T2DM once a day after they are discharged until they recover from COVID-19-like illness and once a week thereafter until Day 56.

Medical history and regular medications will be extracted from the primary care medical records. If participants are hospitalized during the study, clinical data related to COVID-19-like illness will be extracted from the hospital medical records.

**B. Data Points**

- Gut microbiota composition: fecal samples will be collected at Days 0, 28, 56 and the day when participants recover from COVID-19-like illness (if before Day 56)
- Clinical progress of COVID-19-like illness: 1) objective and subjective symptoms data will be collected twice a day from Day 1 to Day 28 or until they recover from COVID-19-like illness (whichever is later) and once a week thereafter until Day 56 (i.e. in the mornings of Days 35, 42, 49 and 56); and 2) COVID-19-like illness severity will be assessed at Days 1, 14, 28 and 56 using the categorization from FDA [18] and the Ordinal Scale for Clinical Improvement from the World Health Organization [19]
- Proportion of participants who are "alive and not admitted to the hospital" at Days 1, 14, 28 and 56
- Proportion of participants who visit the emergency room at Days 1, 14, 28 and 56
- Outcome of COVID-19-like illness (hospitalization or death) at Days 1, 14, 28 and 56
- Glucose homeostasis if the participant has T2DM: fasting blood glucose will be measured in the mornings from Day 1 to Day 28 or until they recover from COVID-19-like illness (whichever is later) and weekly thereafter until Day 56

**C. Study Duration**

It will take each participant 56 days to complete the study. The overall duration of the study is 10 months.

**D. Endpoints**

1. Primary outcome:
   - Gut microbiota composition
2. Secondary outcomes:
   - At Days 1, 14, 28 and 56:

   a) Outcome of COVID-19-like illness (hospitalization or death)
   b) Proportion of participants who are "alive and not admitted to the hospital"
   c) Proportion of participants who visit the emergency room
   d) Proportion of participants who have complete resolution of all objective symptoms, i.e. all vital signs are within the normal range for at least 48 h, including body temperature (oral) 36.5-37.5°C (97.7-99.5°F), pulse rate 60-100/min, respiratory rate 12-20/min and oxygen saturation level at 95-100%
   e) Proportion of participants who have complete resolution of subjective symptoms, i.e. absence of all COVID-19-like symptoms listed by CDC (updated May 13, 2020) for at least 48 h, including the presence of chills or "feeling feverish" , cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting and diarrhea
   f) Proportion of participants who have complete resolution of subjective symptoms except fatigue and cough, i.e. absence of all COVID-19-like symptoms listed by CDC (updated May 13, 2020) for at least 48 h, including the presence of chills or "feeling feverish", cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting and diarrhea
   g) Illness severity based on the categorization from FDA [18] and the Ordinal Scale for Clinical Improvement from the World Health Organization [19]
   h) Body temperature (oral)
   i) Oxygen saturation level
   j) Pulse rate
   k) Respiratory rate
   l) Fasting blood glucose

   - Time to complete resolution of all objective symptoms
   - Time to complete resolution of all subjective symptoms
   - Time to complete resolution of all subjective symptoms except fatigue and cough

### 1.4 Preliminary Data

The work proposed here explores gut microbiota-targeted intervention as an early treatment for COVID-19. Gut microbiota contributes to the host immune homeostasis [20] and has already been implicated in the host's defense against respiratory viral infections [21, 22]. There is also some evidence for interactions between SARS-CoV-2 and the human gastrointestinal system: cell entry of SARS-CoV-2 requires the binding of the viral spike proteins to the angiotensin-converting enzyme 2 (ACE2) and this entry receptor is expressed in the small intestinal enterocytes [23, 24]; viral RNA was detected in 29% (44 of 153) of fecal specimens from a cohort of hospitalized COVID-19 patients and live virus was cultured in a subset of these samples [9]. In one study of 204 patients with COVID-19, almost 20% of the patients exhibited gastrointestinal symptoms (e.g. diarrhea, vomiting and abdominal pain) and these symptoms were more pronounced as disease severity increased [25].

Our intervention to mitigate COVID-19 focuses on promoting a select group of gut bacteria that ferment carbohydrates to produce beneficial SCFAs. In our clinical trials, a diet enriched with non-digestible but fermentable carbohydrates increased SCFA production and contributed to improved metabolic parameters [6, 26]. Importantly, we showed that the predominance of a select group of acetate and butyrate producers was associated with a concomitant reduction in pro-inflammatory and/or endotoxin-producing bacteria. Akin to tall trees in a forest, this group of carbohydrate-fermenting SCFA producers may constitute to a "Foundation Guild" of a healthy gut microbiota structure. In the context of acute respiratory viral infections, Sencio et al [13] showed that mice receiving fecal microbiota transplantation from influenza-infected animals exhibited compromised pulmonary immunity but were protected against bacterial superinfection by acetate supplementation. These data are consistent with our central hypothesis of a SCFA-producing Foundation Guild as the core of a healthy gut microbiota that are protective against complications associated with viral infections.

### 1.5 Sample Size Justification

We will enroll a total of 100 participants who have COVID-19-like symptoms and have positive/pending PCR-based COVID-19 test results. These individuals will be randomized into the control and treatment group in a 1:1 ratio (i.e. 50 participants/group). With the assumption of a positivity rate of 40-50% in symptomatic participants in the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center, we expect to have at least 20 confirmed COVID-19 cases in each group. We used the micropower R-package [27], in which the weighted Jaccard distance matrix was simulated based on within-group distance variance (mean and standard deviation) of data from 36 T2DM patients (unpublished data) to estimate the required sample size. Using the simulated matrices, the PERMANOVA powers are calculated for varying effect sizes ($\omega^2$) and sample sizes. Our results show that 16 subjects per group afford 80% power to an $\omega^2$ of 0.0106 which correspond to the difference ($\omega^2$=0.0120) between the control and treatment group with a similar high-fiber intervention in the T2DM patients (unpublished data). We therefore aim to recruit 20 confirmed COVID-19 cases in each group to allow for potential dropouts and/or incomplete sample/data sets from the participants.

### 1.6 Study Variables

**A. Independent Variables, Interventions, or Predictor Variables**
NBT-NM108 consumption

**B. Dependent Variables or Outcome Measures**
Gut microbiota composition, outcome of COVID-19-like illness (hospitalization or death), proportion of participants who are "alive and not admitted to the hospital", proportion of participants who visit the emergency room at Days 1, 14, 28 and 56, proportion of participants who have complete resolution of all objective symptoms, proportion of participants who have complete resolution of all subjective symptoms, proportion of participants who have complete resolution of all subjective symptoms except fatigue and cough, time to complete resolution of all objective symptoms, time to complete resolution of all subjective symptoms, time to complete resolution of all subjective symptoms except fatigue and cough, COVID-19-like illness severity, body temperature (oral), oxygen saturation level, pulse rate, and respiratory rate

### 1.7 Drugs/Devices/Biologics

Participants in the treatment group will receive NBT-NM108 [redacted] They will be asked to prepare each drink

by mixing one sachet [redacted]. This dosage of NBT-NM108 will provide [redacted] dietary fibers of diverse physicochemical structures as sources of fermentable carbohydrates for the gut microbiota.

**A. Drug/Device Accountability and Storage Methods**

[redacted]

### 1.8 Specimen Collection

**A. Primary Specimen Collection**

- **Types of Specimens**: Each participant will be provided with kits to collect fecal samples at home at each of the following time points: Days 0, 28, 56 and the day when they recover from COVID-19-like illness (if before Day 56). The fecal sample collection kit has been cleared by FDA for at-home use. Instructions and supplies will be provided to collect these samples at home (Appendices 8.1, 8.2 and 16). Participants who have been admitted to the hospital during the study will not collect any samples during hospitalization. If they are discharged on or before Day 56, they will collect a fecal sample at home when they recover from COVID-19-like illness and at Days 28 and 56 as appropriate.

- **Annotation**: Data to be annotated or associated with each specimen will include participant code, age, sex, ethnicity, medical history, and medication.

- **Transport**: Participants will return the samples by mailing them to the research team in prepaid shipping boxes. They will either schedule a FedEx pickup or drop off the package at a local FedEx office. The shipping packages are DOT (Department of Transportation) and IATA (International Air Transport Association) compliant.

- **Processing**: Fecal samples will be processed by Rutgers University research personnel listed in this protocol.

- **Storage**: All samples will be stored in locked -80°C freezers in laboratories in [redacted] and [redacted] ntil analysis. Samples will only be accessible by authorized research personnel named in this protocol. Specimens will be stored until they are no longer in a fit state for scientific analyses.

- **Disposition:** When specimens are no longer in a fit state for scientific analyses, they will be destroyed by incineration by authorized research personnel named in this protocol.

**B. Secondary Specimen Collection**

Not applicable

### 1.9 Data Collection

**A. Primary Data Collection**

- **Location**: *For recruitment at the USF COVID testing center and Rutgers University medical schools*, participants' name and contact details will be obtained from the testing schedules at the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center. Participants' sex, date of birth, race/ethnicity, habitual prebiotics/probiotics consumption, medical history and medications will be collected during the self-administered preliminary screening survey and eligibility screening interview on Castor. *For online recruitment and recruitment through Vault Health company*, individuals who express interest in participating in the study will provide their email addresses in the landing page of the study to access a screening survey and allow communications with the research team. All information necessary for establishing eligibility and confirming enrollment will be collected in the

online self-administered preliminary screening survey and during the informed consent interview. Name and phone number of study candidates will be collected on the HIPPA compliant software Castor if they passed screening survey. Home addresses will be collected over a phone call with the research team. ***During the study***, all participants will record objective and subjective symptoms, adverse events, medication changes, NBT-NM108 (treatment group) and water (control group) consumption at home and report to the research team.

- **Process of Data Collection**: *For recruitment at the USF COVID testing center*, prospective participants will be identified from individuals who have scheduled an appointment at the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center for COVID-19 testing. Study coordinators will call those who appear to be eligible to introduce the study. Potential study candidates who express interest will complete an online self-administered preliminary screening survey (Appendix 2) and then attend an online consent and screening interview with the study coordinator on Castor if they pass the preliminary screening survey. During the eConsent interview, the study coordinator will first confirm that the study candidates continue to experience COVID-19-like symptoms as reported in the preliminary screening survey. Then an electronic informed consent will be obtained and the study coordinator will ask a set of pre-determined questions to collect the patient's medical history and other necessary information to establish eligibility (Appendix 4). Female prospective participants of child-bearing age will be provided with a pregnancy test kit. They will perform the test at home and upload a picture of result to the research team via Castor. *For online recruitment and recruitment through Vault Health company*, individuals who are interested in participating in the study will provide their email addresses on the landing page of the study (Appendix 19) to get access to an online self-administered preliminary screening survey (Appendix 22). They will provide their demographics and medical information for the research team to evaluate the severity of COVID-19-like symptoms. If the study candidates appear to be eligible for the study, they will be instructed to provide their name and phone number on the HIPPA compliant software Castor and schedule an online consent interview. The research team will contact the study candidates over the phone to confirm their details and collect their home addresses to ship study supplies. Female study candidates who are of child-bearing age will be provided with a pregnancy test kit and complete the test prior to the consent interview. During the eConsent interview, the study coordinator will first confirm that the study candidates continue to experience COVID-19-like symptoms as reported in the preliminary screening survey. Then an electronic informed consent will be obtained and the study coordinator will ask a set of pre-determined questions to collect the patient's medical history and other necessary information to establish eligibility (Appendix 4). ***During the study***, all participants will use the provided thermometer, glucometer (if the participant has T2DM), and pulse oximeter to measure body temperature (oral), fasting blood glucose (if the participant has T2DM), pulse rate and oxygen saturation level, record respiratory rate, symptoms, adverse events, medication changes and consumption of NBT-NM108 (treatment group) and water (control group) at home. They will record the data in the participant booklet (Appendices 8.1 and 8.2) and report the data to the research team on Castor (Appendices 9-11).

- **Timing and Frequency**: Prospective participants will complete one online self-administered preliminary survey and attend one virtual eligibility screening interview. All enrolled participants will record objective and subjective symptoms, adverse events and medication changes twice a day (in the morning after at least 8 h of overnight fasting at around 8 am and 2 h after dinner at around 8 pm) and fasting blood glucose (if the participant has T2DM) and intervention compliance once a day from Day 1 to Day 28 or until they recover from COVID-19-like illness (whichever is later). After they recover, they will record these data once a week until Day 56 (i.e. the mornings of Days 35, 42, 49 and 56 as appropriate). Participants will also record these data on the day that they recover from COVID-19-like illness (if before Day 56). If participants are hospitalized during the study, they will discontinue all data collection by themselves during hospitalization but relevant clinical data will be retrieved from the hospital medical records. If participants are discharged on or before Day 56, they will record objective and subjective symptoms, adverse events and medication changes twice a day (in the morning after at least 8 h of overnight fasting around 8 am and 2 h after dinner around 8 pm) and fasting blood glucose (if the participant has T2DM) once a day until they recover from COVID-19-like illness and thereafter record these data once a week until Day 56.

- **Procedures for Audio/Visual Recording**: Not applicable

- **Study Instruments**: Self-administered preliminary screening surveys (Appendices 2 and 22) and a screening checklist (Appendix 4) will be used during the eligibility screening interview to collect information necessary to determine eligibility. A booklet (Appendices 8.1 and 8.2) will be provided to all enrolled participants which will include the study schedule, instructions for using the thermometer, pulse oximeter and glucometer, instructions for intervention, sample collection and shipment. The booklet will also include instructions and tables for participants to record fasting blood glucose (if the participant has T2DM), objective and subjective symptoms, medication changes, adverse events, and intervention compliance. Participants will report these data to the study team on Castor, a cloud-based clinical data management platform (Appendices 9-11). Participants will have access to an interactive online personal dashboard to review their study progress and the data that they have reported (Appendix 17). Participants will have access to a series of videos that provide an overview of the study, how to complete the preliminary screening survey and what is required of them during the study (Appendix 16). A stool sample description form (Appendix 12) will be provided to the participants to describe the fecal sample based on the Bristol Stool Scale [17]. Written step-by-step instructions will be provided for the participants to ship the fecal samples to the research team (Appendix 18).

- **Ethnographic Studies, Interviews, Or Observation**: Not applicable

- **Subject Identifiers**: *For recruitment at the USF COVID testing center and Rutgers University medical schools*, subject identifiers (name, date of birth, phone number, email and home address) will be retrieved from the testing schedules at the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center and testing center at Rutgers University medical schools and collected during the eConsent and eligibility screening interview. *For online recruitment and recruitment through Vault Health company*, individuals who are interested in the study will provide their email addresses on the landing page of the study for the research team to initiate contacts. Date of birth will be collected in the online self-administered screening survey. Name and phone number will be collected on castor and in the HIPPA compliant scheduling software 10To8. Home address will be collected over the phone by the study coordinators if the study candidates appear to be eligible for informed consent and the next stage of screening. This information is only used to establish eligibility and schedule supplies shipment and follow-up phone calls. Participants will provide their social security number as per Internal Revenue Service (IRS) reporting requirements if they agree to receive participant compensation via the ClinCard. No identifiers will be collected in other study instruments.

**B. Secondary Data Collection**

Medical history, medications, clinical data related to and outcome of COVID-19-like illness (hospitalization or death) will be extracted from primary care and hospital medical records.

### 1.10 Timetable/Schedule of Events

We expect to complete this trial within 10 months from contract execution. Detailed timeline is as follows:

| | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Institutional approval, staff training and material development | | | | | | | | | | |
| Recruitment | | | | | | | | | | |
| Intervention, sample measurement and clinical data collection | | | | | | | | | | |
| Bioinformatics and data analysis | | | | | | | | | | |
| Results dissemination | | | | | | | | | | |

## 2.0 Project Management

### 2.1 Research Staff and Qualifications

**University of South Florida**

**Co-PI Dr. Asa Oxner (BSc, MD**) is the Vice Chairman of the Department of Internal Medicine and an Associate Professor at the University of South Florida. Dr. Oxner contributes to the experimental design and will lead the University of South Florida study team to provide logistical and clinical support to the study as study physician.

**Clinical Research Coordinator** [redacted]

**Research Associate** [redacted]

**Rutgers University**

**Co-PI Dr. Liping Zhao (BSc, MSc, PhD)** is the Eveleigh-Fenton Chair of Applied Microbiology in the School of Environmental and Biological Sciences and the Director of Center for Nutrition, Microbiome & Health at New Jersey Institute for Food, Nutrition and Health (IFNH). Dr. Zhao's research applies molecular and genomic tools in understanding systems biology and predictive manipulation of the gut microbial communities, with his recent research primarily focuses on using dietary interventions to restore and maintain a health gut microbiota as a key strategy to improve metabolic health. With extensive experience in using high-fiber diets in clinical trials to modulate the gut microbiota, Dr. Zhao leads experimental design and will contribute to data analysis and interpretation.

**Co-Investigator** [redacted]

**Co-Investigator** [redacted]

**Co-Investigator** [redacted]

**Co-Investigator** [redacted]

**Co-Investigator** [redacted]

**Co-Investigator** [redacted]

**Graduate Students** [redacted]

**Notitia Biotechnologies Company**

**Research Staff** [redacted]

**Vault Health**

**Co-Investigator** [redacted]

### 2.2 Research Staff Training

All investigators have successfully completed the Collaborative Institutional Training Initiative (CITI) training.

### 2.3 Resources Available

Castor, a cloud-based clinical data management platform, and its video call module eConsent, will be used for conducting preliminary screening surveys and virtual interviews for electronic informed consent and eligibility screening, compiling study data and tracking the progress of each participant and the overall study.

Study teams at USF, Rutgers University and Vault Health company will lead the screening, recruitment and enrollment efforts, which include identifying prospective participants, completing questionnaires and administering and/or coordinating tests to establish eligibility. The medical teams at USF and Rutgers University will monitor the clinical progress of participants in Florida and other states respectively, and provide medical support throughout the study, including a 24/7 on-call physician for medical questions related to COVID-19. ClinCard, a participant payment automation system at USF, will be used to provide a stipend for participation for those who are enrolled at the USF COVID testing center, which will be applied to the ClinCard within three working days after completing each specified milestone.

Vault Health is a digital healthcare company and a leading provider of COVID-19 testing and vaccination services in the United States. Vault's solutions have been deployed across 20 different industry segments, including energy, finance, aerospace, pharma/biotech, insurance, state government (e.g. Minnesota, New Jersey, Wyoming, Wisconsin and others), local municipalities, healthcare, recreation (e.g., summer camps), higher education (including athletic programs), and professional sports leagues. To date, Vault has provided COVID testing to over 2 million individuals, providing accurate and timely information in the battle against COVID. Vault is also providing state-sponsored vaccination services in New Jersey, Minnesota, Delaware and elsewhere around the United States. Vault maintains a clinical group practice of over 1,200 practitioners including physicians, advanced practice practitioners (NP / PA), nurses, and medical assistants with licenses spanning all 50 states. Since April of 2020, Vault has facilitated over 4.1 million tests across the United States for the presence of SARS-CoV-2 using the first FDA-authorized saliva-based test. Vault maintains a HIPAA-compliant database of all tests performed with patient information including name, date of birth, race, ethnicity, address / ZIP code, phone number, email address, symptoms of COVID-19 for each test taken, exposure to individuals suspected of or confirmed to have COVID-19 and time since exposure. Vault will utilize this database to identify individuals meeting the below inclusion/exclusion criteria across the United States and utilize "customer relationship management" (CRM) approaches to inform prospective candidates about the study and provide them an opportunity to participate if they meet inclusion criteria.

Fecal sample processing, next-generation sequencing, bioinformatics, and data analysis will take place at the Microbiome Core of New Jersey Institute for Food, Nutrition and Health at Rutgers University as a fee-for-service. Specifically, genomic DNA extraction will be extracted from fecal samples in a biosafety cabinet in a BSL-2 certified laboratory with enhancements. Isolated DNA will then be further processed (e.g. PCR, PCR product purification and dilution) for 16S rRNA V4 amplicon sequencing using the Ion GeneStudio S5 platform (ThermoFisher Scientific). Sequencing data will be analyzed using the Rutgers University high-performance computing cluster Amarel. The Microbiome Core owns a 2X Intel Xeon Skylake 6130 Node (22M Cache, 2.666 MHz, 192 GB RAM, 5 TB storage) of the high-performance computing cluster, which provides the research team high priority and guaranteed access.

### 2.4 Research Sites

University of South Florida:

- Carol & Frank Morsani Center for Advanced Healthcare (13330 USF Laurel Dr, Tampa, FL 33612)
- USF COVID-19 drive through testing site (3515 East Fletcher Avenue, Tampa, FL 33612)

Rutgers University medical schools:

- 90 Bergen Street, Newark, NJ 07103

Robert Wood Johnson Medical School:

- 317 George Street, New Brunswick, NJ 08901

Rutgers University:

- New Jersey Institute for Food, Nutrition and Health (61 Dudley Rd, New Brunswick, NJ 08901)
- Zhao Lab (76 Lipman Dr, New Brunswick, NJ 08901)

Vault Health company:

- 22 W 23rd St, PH, New York, New York 10010

## 3.0 Multi-Center Research

The conduct of the current protocol will be carried out at University of South Florida and Rutgers University.

## 4.0 Subject Considerations

## 4.1 Subject Selection and Enrollment Considerations

### A. Method to Identify Potential Subjects

*For recruitment at the USF COVID testing center and Rutgers University medical schools*, testing schedules at the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center and testing center at Rutgers University medical schools will be screened to identify potential study candidates. Family physicians will be contacted, and flyers will be used to increase advertising exposure. Individuals who call the COVID-19 hotline will also be informed about the study. *For online recruitment*, Google, Facebook and Twitter advertisements will be sent to residents of mainland US. A link to the landing page of the study will also be distributed to family physicians and doctors in the USF and Rutgers University medical schools and facilities via email, text or messaging apps for them to share with their patients and networks. *For recruitment from Vault Health company,* individuals who has COVID-19 related symptoms and has been tested positive for COVID-19 using service from Vault Health company will be contacted by Vault Health. An email containing a brief description of the study and a link to the landing page will be sent to the potential participants.

### B. Recruitment Details

*Recruitment at the USF COVID testing center and Rutgers University medical schools:*

- *Drive-through testing center*: Individuals who have scheduled an appointment at the testing center will be pre-screened. Those appear to be eligible for this study will be contacted by a study coordinator.
- *Referrals from family physicians*: family physicians will be contacted by email (Appendix 13) about this study and provided with recruitment contacts.
- *Flyers*: flyers (Appendix 14) will be posted on the USF campuses, clinics, neighborhoods nearby and social media. Flyers will also be distributed to USF faculties, staff and students via mailing lists.
- *COVID-19 hotline*: individuals who call the COVID-19 hotline will be provided information about this study and contact details of the research team.

*Online recruitment:*

- Individuals who are interested in participating in the study will access the landing page of the study (Appendix 19) either via the Google/Facebook/Twitter advertisements (Appendix 20) or the link shared by their family physicians, USF or Rutgers University. They will register and complete an online self-administered preliminary screening survey. Those appear to be eligible will proceed to an informed consent interview and further screening.

*Recruitment from Vault Health company:*

- Individuals who has COVID-19 related symptoms and has been tested positive for COVID-19 using service from Vault Health company will be contacted by Vault Health. An email containing a brief description of the study and a link to the landing page will be sent to the potential participants. If the study candidate is interested in the study, they will register and complete an online self-administered preliminary screening survey. Those appear to be eligible will proceed to an informed consent interview and further screening.

### C. Subject Screening

- **Inclusion Criteria**
  - Aged between 18 to 79 (inclusive)
  - Have mild to moderate COVID-19-like symptoms based on the symptom list from CDC (updated May 13, 2020) and the severity categorization from FDA [18]:
    a) Mild COVID-19

- Fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea
- No clinical signs indicative of moderate, severe, or critical illness severity

b) Moderate COVID-19

- Symptoms of moderate illness with COVID-19 could include any symptom of mild illness or shortness of breath with exertion
- Clinical signs suggestive of moderate illness with COVID-19, such as respiratory rate ≥ 20 breaths/min, oxygen saturation level > 93% on room air at sea level, heart rate ≥ 90 beats/min
- No clinical signs indicative of severe or critical illness severity

- Directed to home isolation by study physician
-
- Practice acceptable contraception, i.e., continue with current methods if participants are already practicing contraception, otherwise parti cipants must agree to practicing contraception with a barrier method (male or female condom) or abstinence, from Day 1 to Day 35 or 7 days after the last dose of NBT-NM108 if they do not complete the 28-day intervention.
- Have access to a smartphone, tablet, computer, or other qualifying internet-enabled device and daily internet access
- Understand and be able to follow written and oral instructions in English
- Provide informed consent

▪ **Exclusion Criteria**

- Receiving vancomycin monotherapy or oral broad-spectrum antibiotics
- Inability to receive oral fluids
- Self-reported allergy or intolerance to any ingredients in NBT-NM108
- Surgery involving the intestinal lumen within the last 30 days
- Documented diagnosis of celiac disease, inflammatory bowel disease or irritable bowel syndrome
- Pregnancy or breastfeeding
- Bariatric surgery
- Immunocompromised, e.g., cancer treatment, bone marrow/organ transplant, immune deficiency, poorly controlled HIV/AIDS, prolonged use of steroids or other immunosuppressant medications

**4.2 Secondary Subjects**

Not applicable

**4.3 Number of Subjects**

**A. Total Number of Subjects**

100

**B. Total Number of Subjects If Multicenter Study**

N/A

**C. Feasibility**

There are over 2,000 new COVID-19 cases every day in Florida. Around 100 individuals attend the USF Carol & Frank Morsani Center for Advanced Healthcare drive-through testing center for COVID-19 testing each day and among the confirmed cases the prevalence of diabetes is 12%. We therefore consider this patient flow sufficient for recruiting the targeted number of subjects in the specified duration.

**4.4 Consent Procedures**

**A. Consent Process**

▪ **Location of Consent Process**

The electronic consent process will take place during virtual interviews on eConsent, a video call module from the cloud-based clinical data management platform Castor. Individuals who are willing to participate in this study will provide their electronic informed consent during the interview.

- **Ongoing Consent**
  Not applicable

- **Individual Roles for Researchers Involved in Consent**
  Study coordinators will conduct the consent interview to explain all aspects of the study and answer any questions the prospective participants may have.

- **Consent Discussion Duration**
  The consent discussion will take approximately 30 min.

- **Coercion or Undue Influence**
  In order to minimize the possibility of coercion or undue influence, the consent process will not be conducted by research personnel who have financial conflicts of interest or any known relationship or conflict of interest with the prospective participants.

- **Subject Understanding**
  Throughout the consent process the prospective participants will be encouraged to ask any questions should they feel the need to do so. The consent process will be divided into a series of small sections. Members of the research team will summarize each section, use probe questions to check for sufficient understanding before proceeding to the next section.

**B. Waiver or Alteration of Consent Process**

- **Waiver or Alteration Details**
  We are requesting an alteration of the consent process. Prospective participants will be provided with a copy of the consent form (Appendix 3) prior to a virtual consent interview on eConsent, a video call module of the cloud-based clinical data management platform Castor. During this interview, the study coordinator will go through the contents of the consent form with the prospective participants and both parties will provide electronic signatures on the consent form (Appendix 4) if the prospective participants are willing to provide information for eligibility screening. This alteration aims to minimize face-to-face contact between research personnel and COVID-19 patients.

- **Destruction of Identifiers**
  The master code identifier, which contains personal information of subjects and the participant code assigned to them, will be deleted from the server 6 years after study completion.

- **Use of Deception/Concealment**
  Not applicable

**C. Documentation of Consent**

- **Documenting Consent**
  Prospective participants and the study coordinator who conducts the consent process will view and provide electronic signatures on the consent form in real-time on Castor, and both parties will keep a copy of the form.

- **Waiver of Documentation Of Consent (i.e., will not obtain subject's signature)**
  Not applicable

### 4.5 Special Consent/Populations

**A. Minors-Subjects Who Are Not Yet Adults**
Not applicable

**B. Wards of the State**

Not applicable

**C. Non-English-Speaking Subjects**

Not applicable

**D. Adults Unable to Consent / Decisionally Impaired Adults**

Not applicable

### 4.6 Economic Burden and/or Compensation for Subjects

**A. Expenses**

We do not expect any expenses the participants will incur by taking part in the research.

**B. Compensation/Incentives**

Participants will receive compensation of up to $100.00, which will be paid in the form of a USF ClinCard if they are recruited from the USF COVID testing center or in the form of reward cards if they are recruited online. The stipend will be available to the participants within one week after they successfully reach the following milestones:

- $25.00 after the completion of sample collection and shipment at Day 0
- $25.00 after the completion of sample collection and shipment at Day 28
- $25.00 after the completion of sample collection and shipment at Day 56, and an additional $25.00 if the participants successfully complete sample collection and shipment at all time points (including sample collection and shipment at the day of recovery, if applicable)

**C. Compensation Documentation**

Not applicable

### 4.7 Risks of Harm/Potential for Benefits to Subjects

**A. Description of Risks of Harm to Subjects**

- **Reasonably Foreseeable Risks of Harm**

  Measuring fasting blood glucose during the study both involve a finger prick that pierces the skin. There may be a bruise, bleeding, or infection, at the place where the skin is pierced. However, infection is rare. Collecting fecal samples at home may impose inconvenience for the participants and their families. Participants may experience gastrointestinal symptoms (e.g. abdominal pain/discomfort, constipation, or bloating). These discomforts are minor and should not last longer than a few hours. Participants in the treatment group may experience hypoglycemia when they take NBT-NM108 if they are on diabetic medications. This product is low in digestible and simple carbohydrates and may reduce food intake. For participants who are already on diabetic medications (insulin and/or oral hypoglycemic agents) which lower blood glucose, the medication may reduce blood glucose beyond its normal level.

- **Risk of Harm from an Intervention on a Subject with an Existing Condition**

  Not applicable

- **Other Foreseeable Risks of Harm**

  Not applicable

- **Observation and Sensitive Information**

  Not applicable

**B. Procedures which Risk Harm to Embryo, Fetus, and/or Pregnant Subjects**

Not applicable

**C. Risks of Harm to Non-Subjects**

Fecal sample collection at home may impose inconvenience for individuals in the same household.

**D. Assessment of Social Behavior Considerations**
Not applicable

**E. Minimizing Risks of Harm**
Participants will be provided with oral and written instructions and supplies to collect fecal samples at home without direct physical contact with the specimens throughout the process. The specimens will be stored in a sealed container, a leak-proof secondary package (a sealable plastic bag lined with absorbent pads) and an outer package (e.g. a shipping box). Participants are not required to store the samples at home; they will mail the specimens to the research team using the shipping supplies provided.

To minimize adverse effects due to NBT-NM108, participants in the treatment group will be asked to gradually increase the dose [redacted] to avoid adverse events associated with an acute increase in fiber intake. They will also be advised to drink enough fluids throughout the study [redacted] including the amount of water taken together with NBT-NM108) to avoid constipation. Participants will be asked to monitor potential adverse events daily. If participants report an adverse event, a member of the study team will contact them to ask follow-up questions about the degree of severity (mild, moderate, or severe) and the frequency of the adverse events. The study physician (co-PI Dr. Oxner) will assess the adverse events and, if necessary, she will instruct the participants to reduce the consumption of NBT-NM108 to a lower dose that has been tolerated, i.e., [redacted] and so on. If the same level of discomfort continues at [redacted] for three consecutive days, the treatment will be discontinued. Participants are also asked to closely monitor their blood glucose level and discuss with their family physicians about adjusting their medications to prevent hypoglycemia.

To monitor COVID-19-like symptoms, participants will evaluate their symptoms and report to the research team on Castor twice a day from Day 1 to Day 28 or until they recover from COVID-19-like illness (whichever is later) and once a week thereafter until Day 56 (i.e. the mornings of Days 35, 42, 49 and 56 as appropriate). For those who are hospitalized during the study, after they are discharged they will also be asked to evaluate their symptoms and report to the research team on Castor twice a day until they recover from COVID-19-like illness and once a week thereafter until Day 56. The study team will review the symptom diary within 24 h of each entry and follow up with the participants if the symptoms require medical attention. The study team will call all participants at least once a week to monitor their progress. Participants are also instructed to call 911 if they notice any of the following red flags: respiratory rate $\geq$ 30/min, pulse rate $\geq$ 125/min, oxygen saturation level $\leq$ 93%, severe shortness of breath at rest (inability to talk in full sentences), difficulty breathing, persistent pain/pressure in chest, cold/clammy/pale/mottled skin, new confusion or inability to wake up, blue lips or face, little or no urine output or hemoptysis. Participants with T2DM will be instructed to call the physician who normally manages their diabetes if their fasting blood glucose is < 60 mg/dL or > 300 mg/dL.

- **Certificate of Confidentiality**
  Not applicable

- **Provisions to Protect the Privacy Interests of Subjects**
  Should any participants desire not to interact with or provide personal information to specific members of the research team, they may report such a need to the PI and the arrangement of the research personnel will be adjusted accordingly. Participants will not be required to provide a reason if they desire not to do so. Should changing of the research personnel is not possible, the PI will discuss with the subjects about alternative solutions.

  Each participant will be assigned a unique identification code. A master code identifier which links the identification code and personal information will be maintained separately from the study data. Subject information and de-identified study data are only accessible to named investigators in this protocol and authorized research personnel. All data and materials collected during this study are for research

purpose only, and the data will be kept in strict confidence. No information will be given to anyone without permission from the subjects.

**F. Potential Benefits to Subjects**
Participants in the treatment group may have reduced severity of COVID-19-related illness.

## 5.0 Special Considerations

### 5.1 Health Insurance Portability and Accountability Act (HIPAA)
We will ask participants for their consent so that designated members of the research team can review his/her medical record to retrieve medical history, medications, clinical and laboratory data related to COVID-19-like illness. All received health information will be stored in a password-protected file which is stored on the university's secure server and will only be accessible to authorized research personnel.

### 5.2 Family Educational Rights and Privacy Act (FERPA)
Not applicable

### 5.3 Code of Federal Regulations Title 45 Part 46 (Vulnerable Populations)

**A. Special Populations**
Not applicable

### 5.4 General Data Protection Regulation (GDPR)
Not applicable

### 5.5 NJ Access to Medical Research Act (Surrogate Consent)
Not applicable

## 6.0 Data Management Plan

### 6.1 Data Analysis
DNA, protein, and metabolites will be extracted from fecal samples. 16S rRNA gene V4 region sequencing and metagenomic shotgun sequencing with different platforms such as Illumina and PacBio will be done using the extracted DNA. Metaproteome profiling will be achieved using mass spectrometry-based proteome analysis based on extracted protein. Metabolomics will be measured using fecal sample supernatant. Multi-omics data will be combined with clinical data for statistical analysis to explore the overall changes during COVID-19 course and changes induced by the treatment.

16S rRNA gene sequencing data will be analyzed based on the amplicon sequence variants (ASVs) [28] to determine the gut microbiota composition using QIIME 2 [29]. Shannon index and ASV richness will be used to assess alpha diversity. Principal coordinate analysis will be used to compare and visualize dissimilarity in gut microbiota structure between samples based on beta diversity (Weighted and Unweighted UniFrac distance) and statistical differences will be tested using permutational multivariate analysis of variance (PERMANOVA). PICRUSt 2 will be used to predict the functions of individual ASVs and the collective functions of the gut microbiota at the gene content and pathway levels [30]. Global functional profiles of the gut microbiota will be compared and visualized by principal component analysis.

Metagenomic sequencing data will be analyzed in a genome-centric way. The raw sequencing data will be processed with KneadData (https://huttenhower.sph.harvard.edu/kneaddata) for quality control. High quality draft genomes will be de novo assembled from the high-quality sequencing data. The quality assessment, taxonomic assignment and functional annotation of the genomes will be conducted by using CheckM [31], GTDB-Tk [32] and Prokka [33] respectively. Principal coordinate analysis will be used to compare and visualize dissimilarity in gut microbiota structure between samples based on beta diversity and statistical differences will be tested using permutational multivariate analysis of variance (PERMANOVA).

**The metaproteomes will be analyzed by using Unified Human Gastrointestinal Genome (UHGG) v2.0 as the reference for functional annotation and protein sequences database. The predicted protein sequences from metagenomic dataset will be aligned with the measured protein sequences from metaproteomic dataset for integrative analysis.** Global level protein changes of the gut microbiota will be compared and visualized by principal component analysis. **The metabolome will measure both polar metabolites and lipids.** Global level of compound changes of will be compared and visualized by principal component analysis.

Repeat measures correlation will be used to assess the relationships between ASVs/high quality draft genomes [34], followed by ASV/high quality draft genome clustering based on co-abundance patterns. Multivariate methods such as MaAsLin2, that allow adjustment for confounding variables and model the covariates as random effects, will be applied to interpret the relationship between ASVs / ASV co-abundance groups/functions/high quality draft genomes/high quality draft genome co-abundance groups and the clinical metadata/proteins/metabolites.

To analyze the clinical data, Aalen's additive survival model will be used to test whether the impact of covarites on the cumulative hazard is time-dependent [35]. If the covariates do not vary with time, cox proportional hazards model will be subsequently used to adjust for age, gender, race/ethnicity, baseline COVID-19 severity, and whether the participant has taken treatments that may reduce COVID-19 severity (vaccination and monoclonal antibody treatment) as covariates. Survival analysis Kaplan-Meyer curves and log-rank test will be used to estimate and compare the risk of death and the risk of recovery, as a function of time, in participants with COVID-19-like symptoms [36, 37]. To compare the time to hospitalization, the time to recovery, the time to complete resolution of subjective symptoms, and the time to complete resolution of objective symptoms between treatment groups, we will use competing risks survival analysis (treating death or self-reported illness severity at Days 1, 14, 28 and 56 as a competing risk) [36, 38], use survival analysis without competing risk, and explore joint survival analysis and Bayesian survival model [39]. Specifically, cumulative incidence functions will be estimated and tested using Gray's test [40]. The Fine-and-Gray subdistribution hazard regression analysis [38] will be used to further adjust for age, gender, race/ethnicity, baseline COVID-19 severity, and whether the participant has taken treatments that may reduce COVID-19 severity (vaccination and monoclonal antibody treatment) as covariates. Proportion of participants who are "alive and not admitted to the hospital", proportions of participants who visit the emergency room at Days 1, 14, 28 and 56, have complete resolution of objective symptoms, have complete resolution of subjective symptoms and have complete resolution of subjective symptoms except cough and fatigue at Days 1, 14, 28 and 56 will be compared between groups using separate logistic regression analysis at each time point. Time for participants to have complete resolution of objective symptoms, complete resolution of subjective symptoms and complete resolution of subjective symptoms except cough and fatigue will be compared between groups using competing risks survival analysis described previously, treating death or self-reported illness severity at Days 1, 14, 28 and 56 as a competing risk [36, 38]. To compare the longitudinally (repeatedly) measured illness severity based on the Ordinal Scale for Clinical Improvement from the World Health Organization, body temperature (oral), oxygen saturation level, pulse rate, respiratory rate and fasting blood glucose between treatment groups, accounting for the effect of death, reported adverse event, or other appropirate competing risk, we will compare the treatment effect using the method of joint survival and longitudinal data analysis [41, 42], where each of the clinical outcomes (e.g., illness severity, body temperature (oral), and oxygen saturation level, etc.) will be modeled using linear mixed models, and death, reported adverse event, or other appropirate competing risk will be modelled using Weibull proportional hazards model [43]. Age, gender, race/ethnicity, baseline COVID-19 severity, and whether the participant has taken treatments that may reduce COVID-19 severity (vaccination and monoclonal antibody treatment) will be controlled as covariates in these statistical analyses, where appropriate. Within-group changes of main outcomes between groups will be modeled as change from pre- to post-treatment usng ANOVA and modeled as the post-treatment value as the change itself with the pretreatment value entered as covariate using ANCOVA [44]. Lastly, alternative statistical methods will be explored to quantify the underlying differences between groups with respect to a time-to-event end point, robust methods include calculating ratio/difference of t-Year Survival Rates, ratio/difference of percentiles of survival functions, and ratio/differenceof restricted mean survival times or restricted mean time lost [45]. Statistical analyses described above will be conducted for both intent-to-treat dataset and per-protocal dataset. For each test of an outcome, we define the statistical significance by $p<0.05$. The false discovery rate [46] will be applied for multiple testing, where appropriate. Sensitivity analysis using multiple imputation methods [47] will be performed to handle the missing data. Other

approaches, such as methods of selection models [48] or use the pattern-mixture models such as the control-based pattern imputation approach, or the tipping-point approach [49, 50] will also be considered.

### 6.2 Data Security

The study data will include two key components: 1) a master code identifier that contains the personal information of the participants and the participant code assigned to them; and 2) study data files that contain all the de-identified data generated in this trial. Participants' hard copy files will be kept in locked cabinets only accessible by authorized research personnel in a secured building. Electronic data will be stored on Castor, a cloud-based clinical data management platform certified for ISO 27001 (Standards for Information Security Assurance). The data will only be accessible to authorized research personnel, who will have individual accounts that require strong passwords. The master code identifier and the data files will be kept in two separate locations. The master code identifier is only accessible to the Principal Investigator and research personnel who schedule appointments and shipments. Data in hard copies and the master code identifier will be kept for 6 years after study completion, then any hard copies will be shredded and the master code identifier will be permanently deleted from the server. As study data will then exist in de-identified form, the electronic data files will be kept indefinitely.

### 6.3 Data and Safety Monitoring

#### A. Data/Safety Monitoring Plan

A Data and Safety Monitoring Board (DSMB) will approve the protocol before the study is initiated, and meet monthly and after 30% of the treatment group participants complete 14 days of the intervention to review study progress including recruitment, retention, adherence, adverse events and study outcomes, as well as to ensure that participant safety is addressed adequately. Clinical progress of COVID-19-like illness and reports of adverse events will be used to assess the safety of the study.

#### B. Data/Safety Monitoring Board Details

The DSMB will include five USF and Rutgers University faculty members with expertise in medicine, epidemiology and/or statistics who are not directly involved with the study. The Board will meet before the study is initiated, monthly thereafter and after 30% of the treatment group participants complete 14 days of the intervention to review study progress and provide recommendations related to continuing, modifying or terminating the study. Any serious adverse event that is life-threatening or results in death, that is possibly, probably, or definitely related to the research protocol, will trigger an immediate suspension of the research. Specifics of the DSMB, including the responsibilities and scheduling of meetings, are detailed in Appendix 15.

### 6.4 Reporting Results

#### A. Individual Subjects' Results

No individual study results will be released to participants.

#### B. Aggregate Results

All participants will receive aggregate results that summarize the overall findings of this study. The results will be in the form of a one-page summary that will be distributed via email.

#### C. Professional Reporting

Preliminary results will be presented in local and international academic conferences. The final study findings will be published in peer-reviewed academic journals.

#### D. Clinical Trials Registration, Results Reporting and Consent Posting

This study has been registered on ClinicalTrials.gov (NCT04540406).

### 6.5 Secondary Use of the Data

Not applicable

## 7.0 Research Repositories – Specimens and/or Data

Not applicable

## 8.0 Approvals/Authorizations

Notitia Biotechnologies has been issued an Investigational New Drug application to FDA to use NBT-NM108 in the current study (IND # [redacted]). Approval from Institutional Biosafety Committee of Rutgers University for fecal sample processing and microbiota profiling has been obtained under Co-PI Dr. Zhao's program of work titled "Personalized gut microbiota-targeted dietary intervention for patients with type 2 diabetes" at Rutgers University (#17-051).

## 9.0 Bibliography


1. Anderson, R.M., et al., *How will country-based mitigation measures influence the course of the COVID-19 epidemic?* Lancet, 2020. **395**(10228): p. 931-934.
2. Zhou, F., et al., *Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.* Lancet, 2020. **395**(10229): p. 1054-1062.
3. Richardson, S., et al., *Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area.* JAMA, 2020.
4. Wu, Z. and J.M. McGoogan, *Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention.* JAMA, 2020.
5. Yeoh, Y.K., et al., *Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19.* Gut, 2021. **70**(4): p. 698-706.
6. Zhao, L., et al., *Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes.* Science, 2018. **359**(6380): p. 1151-1156.
7. Cavaillon, J.M., *Exotoxins and endotoxins: Inducers of inflammatory cytokines.* Toxicon, 2018. **149**: p. 45-53.
8. Naaber, P., et al., *Translocation of indigenous microflora in an experimental model of sepsis.* J Med Microbiol, 2000. **49**(5): p. 431-439.
9. Wang, W., et al., *Detection of SARS-CoV-2 in Different Types of Clinical Specimens.* JAMA, 2020.
10. Antunes, K.H., et al., *Microbiota-derived acetate protects against respiratory syncytial virus infection through a GPR43-type 1 interferon response.* Nat Commun, 2019. **10**(1): p. 3273.
11. Bradley, K.C., et al., *Microbiota-Driven Tonic Interferon Signals in Lung Stromal Cells Protect from Influenza Virus Infection.* Cell Rep, 2019. **28**(1): p. 245-256 e4.
12. Castillo, P.A.C. and T.W. Hand, *A Little Fiber Goes a Long Way.* Immunity, 2018. **48**(5): p. 844-846.
13. Sencio, V., et al., *Gut Dysbiosis during Influenza Contributes to Pulmonary Pneumococcal Superinfection through Altered Short-Chain Fatty Acid Production.* Cell Rep, 2020. **30**(9): p. 2934-2947 e6.
14. Trompette, A., et al., *Dietary Fiber Confers Protection against Flu by Shaping Ly6c(-) Patrolling Monocyte Hematopoiesis and CD8(+) T Cell Metabolism.* Immunity, 2018. **48**(5): p. 992-1005 e8.
15. Yitbarek, A., et al., *Gut microbiota-mediated protection against influenza virus subtype H9N2 in chickens is associated with modulation of the innate responses.* Sci Rep, 2018. **8**(1): p. 13189.
16. Pocock, S.J. and R. Simon, *Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial.* Biometrics, 1975. **31**(1): p. 103-15.
17. Lewis, S.J. and K.W. Heaton, *Stool form scale as a useful guide to intestinal transit time.* Scandinavian Journal of Gastroenterology, 1997. **32**(9): p. 920-924.
18. Services, U.S.D.o.H.a.H., et al., *COVID-19: Developing Drugs and Biological Products for Treatment or Prevention.* 2020.
19. 2020, W.H.O., *WHO R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis*. 2020, World Health Organization 2020.
20. Rooks, M.G. and W.S. Garrett, *Gut microbiota, metabolites and host immunity.* Nat Rev Immunol, 2016. **16**(6): p. 341-52.
21. Ichinohe, T., et al., *Microbiota regulates immune defense against respiratory tract influenza A virus infection.* Proc Natl Acad Sci U S A, 2011. **108**(13): p. 5354-9.
22. Steed, A.L., et al., *The microbial metabolite desaminotyrosine protects from influenza through type I interferon.* Science, 2017. **357**(6350): p. 498-502.
23. Hoffmann, M., et al., *SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.* Cell, 2020.

24. Hamming, I., et al., *Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis.* J Pathol, 2004. **203**(2): p. 631-7.
25. Pan, L., et al., *Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study.* Am J Gastroenterol, 2020. **In press**.
26. Zhang, C., et al., *Dietary Modulation of Gut Microbiota Contributes to Alleviation of Both Genetic and Simple Obesity in Children.* EBioMedicine, 2015. **2**(8): p. 968-84.
27. Kelly, B.J., et al., *Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA.* Bioinformatics, 2015. **31**(15): p. 2461-8.
28. Callahan, B.J., et al., *DADA2: High-resolution sample inference from Illumina amplicon data.* Nat Methods, 2016. **13**(7): p. 581-3.
29. Bolyen, E., et al., *QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science*. 2018, PeerJ Preprints.
30. Douglas, G.M., et al., *PICRUSt2: An improved and extensible approach for metagenome inference.* bioRxiv, 2019: p. 672295.
31. Parks, D.H., et al., *CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes.* Genome Res, 2015. **25**(7): p. 1043-55.
32. Chaumeil, P.A., et al., *GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database.* Bioinformatics, 2019.
33. Seemann, T., *Prokka: rapid prokaryotic genome annotation.* Bioinformatics, 2014. **30**(14): p. 2068-9.
34. Bakdash, J.Z. and L.R. Marusich, *Repeated Measures Correlation.* Front Psychol, 2017. **8**: p. 456.
35. Martinussen, T., et al., *Estimation of direct effects for survival data by using the Aalen additive hazards model.* 2011. **73**(5): p. 773-788.
36. Putter, H., M. Fiocco, and R.B. Geskus, *Tutorial in biostatistics: competing risks and multi-state models.* Stat Med, 2007. **26**(11): p. 2389-430.
37. Meira-Machado, L., et al., *Multi-state models for the analysis of time-to-event data.* Stat Methods Med Res, 2009. **18**(2): p. 195-222.
38. Fine, J.P. and R.J. Gray, *A Proportional Hazards Model for the Subdistribution of a Competing Risk.* Journal of the American Statistical Association, 1999. **94**(446): p. 496-509.
39. Klein, J.P., et al., *Handbook of survival analysis*. Chapman & Hall/CRC handbooks of modern statistical methods. 2014, Boca Raton, FL: CRC Press.
40. Gray, R.J., *A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk.* The Annals of Statistics, 1988. **16**(3): p. 1141-1154.
41. Tsiatis, A.A. and M. Davidian, *JOINT MODELING OF LONGITUDINAL AND TIME-TO-EVENT DATA: AN OVERVIEW.* Statistica Sinica, 2004. **14**(3): p. 809-834.
42. Rizopoulos, D., *JM: An R package for the joint modelling of longitudinal and time-to-event data.* Journal of Statistical Software (Online), 2010. **35**(9): p. 1-33.
43. Rizopoulos, D., *JM: An R Package for the Joint Modelling of Longitudinal and Time-to-Event Data.* 2010, 2010. **35**(9): p. 33 %J Journal of Statistical Software.
44. French, A., et al., *Multivariate analysis of variance (MANOVA)*. 2008.
45. Uno, H., et al., *Moving beyond the hazard ratio in quantifying the between-group difference in survival analysis.* J Clin Oncol, 2014. **32**(22): p. 2380-5.
46. Benjamini, Y. and D. Yekutieli, *The control of the false discovery rate in multiple testingunder dependency.* 2001. **29 %J The Annals of Statistics**(4): p. 1165-1188, 24.
47. Siddique, J., O. Harel, and C.M. Crespi, *Addressing Missing Data Mechanism Uncertainty using Multiple-Model Multiple Imputation: Application to a Longitudinal Clinical Trial.* Ann Appl Stat, 2012. **6**(4): p. 1814-1837.
48. Molenberghs, G. and G. Verbeke, *A review on linear mixed models for longitudinal data, possibly subject to dropout.* Statistical Modelling, 2001. **1**(4): p. 235-269.
49. Little, R.J.A., *Modeling the Drop-Out Mechanism in Repeated-Measures Studies.* Journal of the American Statistical Association, 1995. **90**(431): p. 1112-1121.
50. Roderick, J.A.L. and Y. Wang, *Pattern-Mixture Models for Multivariate Incomplete Data with Covariates.* Biometrics, 1996. **52**(1): p. 98-111.