Investigation of Choice Alteration of the Gut Metagenome on COVID-19 Severity

Investigation of **CH**oice **A**lteratio**N** of **G**ut metagenom**E** on **COVID-19 Severity**

Short Name: **"CHANGE COVID-19 Severity"**

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ABBREVIATIONS

ACE-I	Angiotensin-converting-enzyme inhibitor
ARB	Angiotensin II receptor blocker
ADR	Adverse drug reaction
AE	Adverse event
DSMB	Data safety monitoring board
eCRF	Electronic case report forms
GFR	Glomerular filtration rate
ICU	Intensive care unit
IV	Intravenous
LAR	Legally authorized representative
LFT	Liver function test
MIC	Minimum inhibitory concentration
NSAIDs	Nonsteroidal anti-inflammatory drug
PI	Principal investigator (a clinician responsible for one site)
RCT	Randomized control trial
SAE	Serious adverse events
S/F	SpO_2/FiO_2 ratio
SOC	Standard of Care
SOFA	Sequential Organ Failure Assessment
SOP	Standard operating Procedure

1.0 Protocol Synopsis

Study Design
Blinded, single-center, placebo-controlled randomized clinical trial

Study Aim
To compare the effect of oral magnesium citrate plus a the probiotic Floranex versus placebo on clinical outcomes, determined by the oxygen area under the curve, among adults with COVID-19 requiring hospitalization.

Primary Outcome
1. Change in COVID Ordinal Outcome Scale (Appendix E) through Day 7 after completion of therapy

Secondary Outcomes
1. Time to recovery, which is the time to the earlier of final liberation from supplemental oxygen or hospital discharge
2. Hospital mortality to 28-day mortality (assessed on Study Day 29 from randomization)
3. Time to discharge to 28 days after treatment
4. Quantitative change in nasal viral titer
5. Change in systemic CD4+ and CD8+ T cell cytokine expression
6. Change in Oxygen AUC of SaO2/FiO2 through Day 7
7. Quantitative change in systemic immune profile
8. Oxygen-free days through Day 28
9. Ventilator-free days through Day 28
10. Vasopressor-free days through Day 28
11. ICU-free days through Day 28
12. Hospital-free days through Day 28

Inclusion Criteria
1. Age ≥18 years
2. Currently hospitalized or in an emergency department with anticipated hospitalization.
3. Symptoms of acute respiratory infection, defined as one or more of the following:
    a. Cough
    b. Fever (> 37.5° C / 99.5° F)
    c. Shortness of breath (operationalized as any of the following: subjective shortness of breath reported by patient or surrogate; tachypnea with respiratory rate ≥22 /minute; hypoxemia, defined as SpO2 <92% on room air, new receipt of supplemental oxygen to maintain SpO2 ≥92%, or increased supplemental oxygen to maintain SpO2 ≥92% for a patient on chronic oxygen therapy).
    d. Sore throat
    e. Anosmia
4. Laboratory-confirmed SARS-CoV-2 infection within 10 days prior to randomization
5. Ability to manage own stool care

Exclusion Criteria
1. Pregnancy
2. Breast feeding

3. Mechanical ventilation
4. Current infectious or noninfectious diarrheal illness
5. Unable to randomize within 21 days after onset of acute respiratory infection symptoms
6. Unable to randomize after hospital arrival
7. Colonic obstruction
8. Unresolved hypovolemia
9. Hypermagnesemia
10. Diagnosis of long QT syndrome
11. Known allergy to magnesium citrate the probiotic Floranexs
12. Unresolved electrolyte imbalance, such as hypokalemia or hypocalcemia.
13. Receipt of >1 dose of magnesium citrate, the probiotic Floranex or any other colonic cleansing agent within 7 days prior to enrollment
14. Inability to receive enteral medications
15. Refusal or inability to be contacted on Day 7 for clinical outcome assessment if discharged prior to Day 7
16. Concurrent medical illness that interferes with clinical assessments
17. The treating clinical team does not believe equipoise exists regarding the use of magnesium citrate plus a the probiotic Floranex for the treatment of this patient
18. Immunologically vulnerable patients, such as AIDS patients or neutropenic patients
19. Participating in any other COVID-19 therapeutic trials.

Randomization

Eligible participants will be randomized 1:1 to magnesium citrate plus the probiotic Floranex versus placebo. Placebo will be carbonated lemon water for magnesium citrate and over-encapsulated microcrystalline pills for Floranex. Randomization will be completed in permuted blocks of variable size.

2. TRIAL DESCRIPTION

2.1 Background

Coronavirus Disease 2019 (COVID-19) is an acute respiratory infectious illness caused by *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2).[1,2] Although the epidemiology has not been fully elucidated, most adults with COVID-19 appear to experience fever, cough, and fatigue and then recover within 1-3 weeks. However, a portion of adults with COVID-19 develop severe illness, typically manifesting as pneumonia and hypoxemic respiratory failure, with continued progression to acute respiratory distress syndrome (ARDS) and death in some cases.[1–3] The incidence and mortality of COVID-19 cases continues to climb in the United States, as well as around the world. While progress for vaccination and identifying therapeutics against SAR-CoV-2 have emerged, the limitations continue to emerge. For example, convalescent plasma was recently demonstrated to hold little promise against COVID-19 severe pneumonia[1]. While remdesivir demonstrates efficacy in hospitalized COVID-19 patients with pneumonia[2], the NIH no longer recommends remdesivir with dexamethasone as an option for hospitalized COVID-19 patients who require mechanical ventilation or ECMO, recommending only dexamethasone instead (COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 12/07/2020). Thus, data on the safety and effectiveness of magnesium citrate plus the probiotic Floranex for the treatment of COVID-19 are urgently needed to inform clinical practice. In this trial, we will evaluate the safety and effectiveness of magnesium citrate plus the probiotic Floranex for the treatment of adults hospitalized with COVID-19.

2.1.1 COVID-19 Infection

COVID-19 was first identified as a cluster of cases of pneumonia among a group of workers from a seafood wholesale market in Wuhan, China in December 2019.[7] This observation, along with subsequent viral genotyping showing significant genetic similarities to the bat coronaviruses[8] suggest a zoonotic origin, although the specific reservoir and intermediary species remain unclear.[9] The COVID-19 infection represents the seventh coronavirus known to cause disease in humans.[10] Four of the coronaviruses viruses are known to cause symptoms of the common cold in immunocompetent individuals while two others (SARS-CoV and MERS-CoV) have caused recent outbreaks of severe and sometimes fatal respiratory diseases.[11] SARS-CoV-2 appears to exploit the same cellular receptor as SARS-CoV and MERS-CoV,[12] and its severity may similarly result from a predilection for intrapulmonary epithelial cells over cells of the upper airways.[13,14] Since the first documented human case, COVID-19 has spread exponentially within the United States with 14,823,129 confirmed cases and 282,785 deaths as of December 8, 2020. While most patients recover after a mild, brief illness with fever and cough, the disease has a clinical spectrum ranging from asymptomatic infection[15] to ARDS and death.[16] The most common reasons for ICU care are respiratory failure and ARDS, with a minority developing shock and possibly cardiomyopathy.[17] The case fatality rate is estimated to be 0.25% to 3.0%.[18]

2.1.2 Magnesium citrates as a Therapeutic for COVID-19

Recent recommendations, such as the cessation of remdesivir in intubated COVID-19 patients with severe pneumonia, illustrates as important clinical observation: much of COIVD-19 mortality occurs after the virus has been cleared. Viral clearance typically occurs 9-20 days after symptom onset[3]. A similar monkey model of SARS-CoV infection in the African Green Monkey demonstrated clearance of virus from nasal turbinate, nasopharynx, tracheal, proximal and distal lungs within 5-10 days after infection[4]. Similarly, investigators reported that viral loads in SARS-CoV-2–challenged rhesus macaques remain elevated for 10-28 days following primary challenge[5].

A common clinical factor found in persons at risk for COVID-19 severity, such as aging, obesity, diabetes and hypertension, is gut dysbiosis and resulting low grade inflammation with loss of epithelial barrier function[6, 7]. A recent study noted that compared with healthy controls, COVID-19 patients had significantly reduced bacterial diversity, a significantly higher relative abundance of opportunistic pathogens, such as *Streptococcus, Rothia*, *Veillonella* and *Actinomyces,* and a lower relative abundance of beneficial symbionts[8, 9]. Another investigation

of faecal samples with signature of high SARS-CoV-2 infectivity had higher abundances of bacterial species, such as *Collinsella aerofaciens, Collinsella tanakaei, Streptococcus infantis, Morganella morganii,* and higher functional capacity for nucleotide de novo biosynthesis, amino acid biosynthesis and glycolysis, whereas faecal samples with signature of low-to-none SARS-CoV-2 infectivity had higher abundances of short-chain fatty acid producing bacteria, *Parabacteroides merdae, Bacteroides stercoris, Alistipes onderdonkii* and *Lachnospiraceae* bacterium in the stool of COVID-19 patients[10]. These observations support assessing for host metagonomic contributors to COVID-19 morbidity and mortality.

Alterations of the microbiota that lead to intestinal dysbiosis (a microbial imbalance within the intestinal tract) are characterized by a loss or significant decrease in the amount of beneficial bacterial species and/or an outgrowth or population shift of pathogenic species. A growing body of literature implicates the role of the gut microbiome in the severity of chronic viral infections. Perturbation of the gut microbiota during influenza A virus (IAV) infection might favor respiratory bacterial superinfection[11]. Alterations in the gut microbiome itself may affect influenza severity. In general, mice treated with broad spectrum antibiotics display diminished innate and adaptive immune responses, characterized by reduced expression of antiviral genes in alveolar macrophages, more severe bronchiole epithelial degeneration, and higher viral loads compared to untreated mice[12]. Laboratory mice reconstituted with natural microbiota exhibited reduced inflammation and increased survival following influenza virus infection and improved resistance against mutagen/inflammation-induced colorectal tumorigenesis[13]. It has been suggested that gut microbiota can fine-tune the threshold of antiviral immune responses[14], possibly through metabolites such as short chain fatty acids (SCFAs). The critical role of SCFAs in the gut-lung axis is well-recognized, and studies in a mouse model have shown that consumption of SCFAs reduces inflammatory symptoms, and also decreases allergenic diseases in the airway though suppression by regulatory T (Treg) cells[15]. Indeed independent reports suggest that alteration of the gut microbiome may positively affect COVID-19 severity. Suggestions, such as the probiotic Floranex and high fiber diets, have been postulated to reduce COVID-19 severity[16]; however, that ability of such interventions to rapidly affect alter the gut microbiome in hospitalized patients, and thus potentially reduce COVID-19 severity, are limited.

Recent investigations of chemical induction of acute lung inflammation demonstrates significant reductions in lung injury among mice housed in germ-free environments (Dr. Wonder Drake, manuscript in preparation). Also, administration of colonic cleansing via magnesium citrate in three hypoxic, COVID-19 patients with pneumonitis resulted in significant clinical improvement within 24-72 hours (Dr. Wonder Drake, manuscript in preparation).

2.1.3 Rationale for a Randomized Trial among Hospitalized Patients

The initial symptoms of COVID-19 develop approximately 2-10 days after infection with the SARS-CoV-2 virus,[23] with the progression to respiratory failure and ARDS occurring approximately 7-10 days after the onset of symptoms.[24] While most adults with COVID-19 recover without complications, patients who require hospitalization experience high rates of complications. In case series of hospitalized patients with COVID-19, up to 26% require ICU admission and up to 17% die in the hospital.[24,25] The period between onset of symptoms and development of severe respiratory failure represents a potential window for treatment of hospitalized patients to prevent disease progression.

Given the unprecedented public health crisis caused by COVID-19, there is significant interest in finding effective therapies and, specifically, in repurposing approved medications with widespread availability and known safety profiles.[3,26]

Data on the safety and efficacy of magnesium citrate from randomized trials is urgently needed. A randomized clinical trial demonstrating that magnesium citrate prevents disease progression in hospitalized patients with COVID-19 would provide evidence-based therapy for an ongoing pandemic, as well as important public health impact.

Given the need for effective treatments of COVID-19 and the unclear efficacy and safety of magnesium citrate as a treatment of COVID-19a randomized clinical trial is urgently needed.

2.1.4. Rationale for Evaluating Magnesium Citrate in addition to standard therapy

Current therapeutics for COVID-19 include remdesivir, with the caveat of therapeutic limitations among patients who are intubated or on ECMO. While we propose the use of magnesium citrate and the probiotic Floranex in

non-ICU, COVID-19 subjects, any observed clinical benefit will subvert disease progression. During the design of this protocol, the investigators propose to add Magnesium citrate in addition to current therapy. Current COVID-19 therapeutics will not interfere with the therapeutic benefit of magnesium citrate, and fortunately, the converse is also true. Interpretation of a trial of one agent will be straightforward and may provide the basis for subsequent trials of patients with more severe COVID-19 manifestations.

2.2 Study Aims

2.2.1 Study aim

To compare the efficacy of magnesium citrate plus the probiotic Floranex versus placebo on physiology, immunologic and clinical outcomes among adults hospitalized due to COVID-19 infection.

2.2.2 Study hypothesis

Among adults hospitalized with COVID-19, administration of magnesium citrate plus the probiotic Floranex will improve clinical outcomes by Day 28.

2.3 Study Design

We will conduct an investigator-initiated, single center, blinded, placebo-controlled, randomized clinical trial evaluating magnesium citrate plus the probiotic Floranex for the treatment of adults hospitalized with COVID-19. Patients, treating clinicians, and study personnel will all be blinded to study group assignment.

2.4 Outcomes

Primary Outcome

1. Change in WHO Clinical Progression Scale through Day 7 after completion of therapy

Secondary Outcomes

1. Time to recovery, which is the time to the earlier of final liberation from supplemental oxygen or hospital discharge
2. Hospital mortality to 28-day mortality (assessed on Study Day 29 from randomization)
3. Time to discharge to 28 days after treatment
4. Quantitative change in nasal viral titer
5. Change in systemic CD4+ and CD8+ T cell cytokine expression
6. Change in Oxygen AUC of SaO2/FiO2 through Day 7
7. Quantitative change in systemic immune profile
8. Oxygen-free days through Day 28
9. Ventilator-free days through Day 28
10. Vasopressor-free days through Day 28
11. ICU-free days through Day 28
12. Hospital-free days through Day 28

3. STUDY POPULATION AND ENROLLMENT

3.1 Inclusion Criteria

1. Age ≥18 years
6. 2. Currently hospitalized or in an emergency department with anticipated hospitalization.
7. 3. Symptoms of acute respiratory infection, defined as one or more of the following:
    a. Cough

b. fever (> 37.5° C / 99.5° F)
c. shortness of breath (operationalized as any of the following: subjective shortness of breath reported by patient or surrogate; tachypnea with respiratory rate ≥22 /minute; hypoxemia, defined as SpO2 <92% on room air, new receipt of supplemental oxygen to maintain SpO2 ≥92%, or increased supplemental oxygen to maintain SpO2 ≥92% for a patient on chronic oxygen therapy).
d. sore throat

4. Laboratory-confirmed SARS-CoV-2 infection within 10 days prior to randomization

Exclusion Criteria

1. Prisoner
2. Pregnancy
3. Breast feeding
4. Current infectious or noninfectious diarrheal illness
5. Unable to randomize within 21 days after onset of acute respiratory infection symptoms
6. Unable to randomize after hospital arrival
7. Colonic obstruction
8. Unresolved hypovolemia
9. CrCl <30ml/min
10. Hypermagnesemia
11. Diagnosis of Long QT syndrome
12. Known allergy to magnesium citrate or the probiotic Floranex
13. Unresolved electrolyte imbalance such as hypokalemia or hypocalcemia. The patient can be enrolled if electrolytes are corrected and sustained.
14. Receipt of >1 dose of magnesium citrate or any other colonic cleanser in the 7 days prior to enrollment
15. Inability to receive enteral medications
16. Refusal or inability to be contacted on Day 15 for clinical outcome assessment if discharged prior to Day 15
17. Concurrent medical illness that interferes with clinical assessmentPrevious enrollment in this trial
18. The treating clinical team does not believe equipoise exists regarding the use of magnesium citrate plus the probiotic Floranex for the treatment of this patient.
19. Participating in any other COVID-19 therapeutic trial

3.3 Justification of Exclusion Criteria

The exclusion criteria are primarily designed for patient safety. In addition to excluding specific vulnerable populations (e.g., prisoners), these criteria are designed to exclude patients for whom receipt of magnesium citrate plus a the probiotic Floranex might increase the risk of serious adverse events.

3.4 Screening

The site investigator or delegate will screen for hospitalized patients with laboratory confirmed COVID-19 (that is, a positive laboratory test for SARS-CoV-2) or a pending SARS-CoV-2 test. Treating clinicians will also be instructed to contact the site investigator or delegate for patients with a high clinical suspicion of COVID-19.

3.5 Assessment of Eligibility and Exclusion Tracking

For patients who appear to meet inclusion criteria during screening, an electronic case report form will be completed to determine eligibility and track exclusions. The electronic case report form will be accessed and

stored in the electronic database. At the time of entry into the screening database, the patient will be assigned a screening number.
If a patient appears to meet all eligibility criteria, the site investigator or delegate will approach the treating clinician to ask permission to approach the patient or Legally Authorized Representative (LAR) to confirm eligibility, discuss potential study recruitment, and proceed with informed consent.
For all excluded patients, including refusal by the treating clinician or patient/surrogate, a small number of de-identified variables will be collected including month and year the patient met screening criteria, age, sex, ethnicity, patient location, and reason(s) patient was excluded. For the safety of research personnel and conservation of personal protective equipment, these encounters may occur via telephone or videophone.

3.6 Process of Obtaining Informed Consent

Informed consent will be obtained from the patient or from a surrogate decision maker if the patient lacks decision-making capacity.
In some instances, bringing a paper consent form and pen to the bedside of a patient with known or suspected COVID-19 and then taking these out of the room would violate infection control principles and policies. Given the infectious risk from COVID-19 and potential shortages of personal protective equipment (PPE), there is a moral and practical imperative to minimize face-to-face contact between patients and non-clinical personnel. The current epidemic also presents unique challenges to obtaining consent from participant's legally authorized representative (LAR). To minimize infectious risk, many institutions are not allowing visitors to enter the hospital. Furthermore, the LAR is likely to have been exposed to the patient and may therefore be under self-quarantine at the time of the informed consent discussion.
Therefore, in addition to the traditional approach of an in-person consent discussion and signed paper informed consent document, we will allow use of "no-touch" consent procedures for this trial. Below, we outline three examples of no-touch consent procedures that may be used: (a) a paper-based approach; (b) attestation of informed consent.

3.6.1 Paper-based approach

1. The informed consent document is delivered to the patient or LAR.
    a. If the patient or LAR is on-site, the informed consent document many be delivered to the patient or LAR either by research staff or by clinical staff
    b. If the LAR is off-site, the informed consent document may be emailed, faxed, or otherwise electronically transferred to the LAR (method dictated by institutional policy)
2. Research staff discuss the informed consent document with the patient or LAR either in-person or by telephone or videophone. *This step confirms subject/LAR identity*.
3. If the patient or LAR decides to consent to participate, the patient or LAR signs the paper copy of the informed consent document.
4. A photograph is taken of the signature page of the informed consent document and uploaded into the electronic database (e.g. REDCap).
    a. If using the patient's device (such as a patient's personal cellular phone), a survey link can be sent to their device to allow direct upload of the image into the electronic database (e.g. REDCap).
    b. If using a staff device, it must be approved to store PHI by the local institution. In that case, research personnel can take a photograph of the signature page of the informed consent document either directly or through the window or glass door leading into the patient's room. The photograph can then be uploaded into the electronic database. If a staff device is taken into the patient's room to take a photograph it must be able to be disinfected according to local institutional practices.
5. Research staff and witness provide signatures within the electronic database (e.g. REDCap) confirming their participation in the informed consent process.

6. The patient or LAR retains the paper consent document. The image of the signature page may be printed and bundled with a copy of the blank informed consent document for research records.

If a hospital device is provided to facilitate electronic or paper-based consent, that device will be disinfected according to institutional protocols and removed by research staff or clinical staff during the next entry into the patient's room.
This approach complies with relevant regulations and sub-regulator guidance at 45 CFR 46.117, 45 CFR 164.512, 21 CFR 11 Subpart C (11.100–11.300), https://www.hhs.gov/ohrp/regulations-and-policy/guidance/use-electronic-informed-consent-questions-and-answers/index.html, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/informed-consent
The information for the informed consent discussion will be provided in a formal document (or electronic equivalent) that has been approved by the IRB and in a language comprehensible to the potential participant, using an interpreter if necessary. The information presented in the consent form and by the research staff will detail the nature of the trial and what is expected of participants, including any potential risks or benefits of taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. Where a patient does not speak English, a short-form consent and qualified interpreter will be employed, using similar "no-touch" principles. Use of an interpreter and the interpreter's identity will be documented on the electronic consent.

3.7 Randomization and Blinding
Participants confirmed to meet all eligibility criteria who have provided informed consent will be randomized 1:1 to magnesium citrate plus a the probiotic Floranex versus placebo. A randomization code will be provided to the site investigator or delegate from a centralized, web-based platform. Randomization will require provision of the screening number and confirmation of patient eligibility.
Randomization will be completed in permuted blocks of varying size and stratified by site. The randomized sequence allocation will be stored on a secure server and will not be available to site study personnel. Site research personnel will have a unique Personal Identification Number (PIN) to access the randomization system. Each subject will receive a computer-generated randomization ID number. The computer-generated randomization ID number will be provided to the pharmacy who will provide a dose pack containing magnesium citrate plus a the probiotic Floranex or placebo. The participant, treating clinicians, study personnel, and outcome assessors will all remain blinded to group assignment until after the database is locked and blinded analysis is completed.

3.8 Minorities and Women
No patients will be excluded on the basis of race, ethnicity, or sex. The clinical coordinating center will monitor recruitment of minorities and women. If necessary, additional recruitment efforts will be made to ensure that the aggregate patient sample contains representative race/ethnicity and sex subsets.

4. STUDY INTERVENTIONS

4.1 Treatment of Study Participants
A summary of the trial's schedule of events is included in Appendix A.
Timing of study procedures is based on the time of randomization, which is defined as "Time 0". The primary outcome will be assessed on Study Day 7, which corresponds to 7 days after randomization.
Study medications will be administered by clinical or research personnel while the patient is hospitalized. The study medication will be administered within 4 hours of randomization. On Study Day 1, study personnel will review patient records to confirm administration of the study drug and document the volume of magnesium citrate

and the probiotic Floranex was consumed. Research personnel will assess patients at Day 1-5, 7 and through Day 29; these assessments will be completed by phone if the patient has been discharged from the hospital.

4.2 Study Arms

Magnesium Citrate plus The probiotic Floranex Arm:

Participants assigned to the magnesium citrate plus the probiotic Floranex arm will receive 1 bottle of magnesium citrate 296 mL PO once to be taken within a 4-hour period, (about 10 oz.). Because magnesium citrate remains within the intestinal lumen, a second bottle can be provided if there is limited bowel movement. Patients will be asked to take 2 capsules of the probiotic Floranexs twice daily for six days or until discharge, whichever is earlier.

Placebo Arm

Participants randomized to the placebo arm will receive matching carbonated lemon water 296 mL PO once to be taken within a 4-hour period, (about 10 oz.) and 2 over-encapsulated microcrystalline pills capsules twice daily for six days or until discharge, whichever is earlier. The placebo will be flavored to match the taste of the interventional arm.

4.4 Co-Interventions

This trial will control the use of magnesium citrate plus the probiotic Floranex vs placebo during the intervention period. Enrolled participants will not receive open-label magnesium citrate or the probiotic Floranex during the intervention period. All other treatment decisions will be made by treating clinicians without influence from the protocol. Administration of other antiviral medications ("rescue therapy") will be allowed. The decision to administer other antiviral medications will be made by treating clinicians and will be recorded in the case report form. The decision to administer immunomodulating medications, including corticosteroids, will be made by treating clinicians and will be recorded in the case report from.

4.5 On-Study Monitoring

All patients enrolled in the study will be initially hospitalized and will therefore receive monitoring as a part of routine clinical care, including monitoring by their physicians, nurses, respiratory therapists, and ancillary staff.

Study personnel will review the electronic health record daily for new potential medication interactions with magnesium citrate (see Appendix B). If a medication that is considered to be contraindicated with magnesium citrate or the probiotic Floranexs is discovered, treating clinicians will be contacted to discuss if stopping study drugs is appropriate or if the medication in question can be stopped or substituted. If a medication with a potential interaction with magnesium citrate or the probiotic Floranex is identified, study personnel will contact treating clinicians to ensure they are aware of the potential interaction. Treating clinicians will determine whether an alternative medication would be appropriate or whether the risk-benefit ratio favors continuing the medication with the known potential interaction.

In addition to manual monitoring by study personnel for medication interactions, many electronic health records contain tools within the electronic order entry system to automatically screen for medication interactions and notify ordering providers of the potential interaction at the time of order entry.

4.6 Criteria for Stopping Study Drug

Administration of the blinded study drug may be stopped temporarily or permanently for (a) adverse events, (b) results of on-study monitoring, (c) clinical deterioration, or (d) evidence of an alternative cause to the patient's symptoms.

If a patient experiences an adverse event that the patient (or legally authorized representative), treating clinicians, or investigators feel merits temporarily or permanently stopping the study drug, the study drug will be stopped. The explanation for stopping the study drug will be recorded in the case report form, and the adverse event will be recorded and reported according to the adverse event guidelines below. If the adverse event resolves to the extent that the patient (or legally authorized representative), treating clinicians, and investigators feel that resuming the

study drug is appropriate, the study drug will be resumed, and this information will be recorded in the case report form.

Patients on study may experience clinical deterioration due to their illness. Clinical deterioration will be defined as a decrease of 1 point or more on the ordinal scale for the primary outcome (e.g., patient transitions from "hospitalized on supplemental oxygen" to "hospitalized on non-invasive ventilation or high flow nasal cannula"). Patients who experience clinical deterioration in either group may be administered other antivirals or immunomodulators as "rescue therapy". For patients who experience clinical deterioration for which treating clinicians feel optimal care would be to stop the study drug, unblind group assignment, and administer magnesium citrate and/or the probiotic Floranex to patients in the placebo group, the study drug will be stopped, the site investigator will contact the coordinating center to receive the unblinded study group assignment, and any additional treatment will be deferred to treating clinicians. In this situation, the following data will be recorded in the case report form: the criteria met for clinical deterioration; the reason for stopping study drug and unblinding; use of magnesium citrate, other antivirals, and immunomodulators; and study outcomes.

5. OUTCOMES

5.1 Primary Outcome

1. Change in WHO Clinical Progression Scale through Day 7 after completion of therapy

5.2 Secondary Outcomes

1. Time to recovery, which is the time to the earlier of final liberation from supplemental oxygen or hospital discharge
2. Hospital mortality to 28-day mortality (assessed on Study Day 29 from randomization)
3. Time to discharge to 28 days after treatment
4. Quantitative change in nasal viral titer
5. Change in systemic CD4+ and CD8+ T cell cytokine expression
6. Change in Oxygen AUC of SaO2/FiO2 through Day 7
7. Quantitative change in systemic immune profile
8. Oxygen-free days through Day 28
9. Ventilator-free days through Day 28
10. Vasopressor-free days through Day 28
11. ICU-free days through Day 28
12. Hospital-free days through Day 28

5.3 Safety outcomes

- Dehydration
- Symptomatic Hypotension
- Acute kidney injury
- Receipt of renal replacement therapy

5.4 Rationale for Primary Outcome

COVID-19 has a broad spectrum of clinical severity. Even among hospitalized patients, most recover without experiencing critical illness.[30] Designing a trial with statistical power to detect a meaningful difference in ICU-free days or mortality might require an unfeasibly large sample size and could miss significant morbidity experienced by the majority of hospitalized patients. Since the majority of morbidity from COVID-19 relates to hypoxemia, the fact that this outcome is tied to degree of hypoxemic respiratory failure increases its face validity and relevance. For similar reasons, previous trials of severe influenza have employed a similar ordinal outcome.[31]

This ordinal scale has been selected as an outcome in multiple ongoing COVID-19 trials and is a preferred outcome by the World Health Organization Research and Development Blueprint for COVID-19.[32] Use of this standardized outcome will increase the potential to compare the results of this trial with other trials and perform meta-analyses.

6. STUDY ASSESSMENTS, PROCEDURES AND DATA COLLECTION

Given the infectious risk from COVID-19 and potential shortages of personal protective equipment (PPE), we will minimize face-to-face contact between patients and non-clinical staff. Additionally, minimizing research activities and conducting the trial in a pragmatic manner will increase the ability to complete the trial in the face of strained clinical and research resources during the COVID-19 pandemic. We will emphasize data that can be collected from the electronic health record, radiographs obtained as part of routine clinical care, and assessments that can be completed over the telephone as needed.

6.1 Baseline Assessments and Procedures: Hospital Presentation to Randomization (Day 1)

- Inclusion/exclusion criteria screening
- Pregnancy test (if applicable)
- Informed consent
- Completion of COVID Ordinal Outcomes Scale
- Blood collection for CD4+ and CD8+ T cell cytokine expression
- Nasal Swab testing for SARS-CoV-2
- Completion of the Sequential Organ Failure Assessment (SOFA)[33]
- Evaluation of Acute Respiratory Distress Syndrome (ARDS) by Berlin Criteria[33]
- Baseline Variable Data Collection:
  - Hospital admissions data: date/time of presentation, origin (home, skilled nursing facility, rehabilitation/LTACH, nursing home, outside hospital, outside ICU), location at enrollment (ED, hospital ward, ICU
  - Demographics (age, sex, race, ethnicity, height, weight)
  - Signs and symptoms/onset date including acute signs/symptoms such as altered mental status, acute hypoxemic respiratory failure, liver function tests, renal function, coagulation studies, SOC chest imaging results
  - Concomitant Medications/Medication History to include:
    - Chronic use of medications such as: corticosteroids, ACE inhibitors, angiotensin receptor blockers, non-steroids or anti-inflammatory drugs
    - Open label antivirals received such as remdesivir
    - Open label immunomodulators
    - Biologic therapy such a convalescent plasma
  - Comorbidities
  - Oxygen/ventilation assessment including S/F ratio, ventilation type, & vasopressor use (if applicable)
  - Vital signs, including highest respiratory rate, lowest systolic blood pressure, and highest heart rate in the 12 hours prior to enrollment (if available)
- Enrollment/Randomization
- First study drug administration

6.2 Study Assessments/Procedures: Randomization to Hospital Discharge (Daily)

- Study Variable Data Collection
  - Medication Assessment
  - Assess for clinical diagnosis of deep vein thrombosis (DVT) or pulmonary embolism (PE)
  - Oxygen/Ventilation assessment including S/F ratio, ventilation type, & vasopressor use (if applicable)
  - ARDS Assessment by Berlin Diagnostic Criteria (if applicable)
  - ICU admission/discharge information (if applicable)
  - Death date (if applicable)
  - Safety Outcomes: Dehydration, Seizure, Atrial or ventricular arrhythmia, Cardiac arrest, Symptomatic Hypotension, Acute kidney injury, Receipt of renal replacement therapy, Symptomatic hypoglycemia
  - Hospital discharge including patient destination at discharge

6.2.1 Day Specific Assessments: Randomization to Hospital Discharge

6.2.1.1 Days 2-7 (Daily)

- COVID Ordinal Outcomes Scale
- Study Drug Adherence Assessment (days 1-6)
- Assessment of Antibiotic Use including azithromycin

6.2.1.2 Day 3

- Completion of SOFA assessment
- Nasal swab for SARS CoV-2 testing

6.2.1.3 Day 7

- Blood collection for CD4+ and CD8+ T cell cytokine expression

6.3 Assessments following Hospital Discharge

6.3.1 Acute Care Follow-up

For participants discharged from the study hospital prior to the Day 7 or Day 29 assessment, we will perform these assessments via telephone follow-up. The Day 7 call window will be Day 7 through day14. The Day 29 call window will be Day 29 through day 36. During these telephone calls, we will interview the patient, LAR, or facility staff to assess:

- Date of death (if applicable)
- ED visits, hospital readmissions, and use of supplemental oxygen after hospital discharge
- Non-laboratory safety outcomes after hospital discharge and adverse events
- Symptoms of acute respiratory infection
- COVID Ordinal Outcomes Scale

6.3.2 Long-term Follow-up

We will follow-up selected patients at 3months to assess vital status, cognition, basic and instrumental activities of daily living, quality of life, employment status, physical disability, and psychological distress (i.e., depression, post-traumatic stress disorder, etc.), place of residence, and rehospitalizations. These assessments may occur by phone, in-person, or videoconferencing.

7. STATISTICAL CONSIDERATIONS

7.1 Statistical Approach

The primary analysis will be an intention-to-treat comparison of the Day 7 COVID Ordinal Outcome score between patients randomized to magnesium citrate plus a the probiotic Floranex versus placebo. This analysis will be conducted with a proportional odds model using the Day 7 COVID Ordinal Outcome score as the dependent variable, randomized group assignment as the primary independent variable, and the following co-variables: age, sex, baseline COVID Ordinal Outcome score, baseline SOFA score, and duration of acute respiratory infection symptoms prior to randomization. An odds ratio >1.0 indicates more favorable outcomes with magnesium citrate plus the probiotic Floranex on the COVID Ordinal Outcome scale, while an odds ratio <1.0 indicates more favorable outcomes with placebo.

In additional to reporting data for the full trial population we will also report data separately for patients randomized in the ICU (who tend to be more severely ill) and those randomized outside the ICU (who tend to be less severely ill) as well as those with duration of symptoms ≤5 days prior to randomization and those with >5 days of symptoms prior to randomization.

The anticipated study size is 30 patients. We calculated the sample size under the assumption that we would have an interim analysis after approximately each 10 patients. We calculated the standard error of the log(odds-ratio) statistic with 15 patients per arm based on data from a recently completed trial within the PETAL Network that enrolled patients early in the course of critical illness, the *Vitamin D to Improve Outcomes by Leveraging Early Treatment* (VIOLET) trial.[35] In the VIOLET trial at Day 15, 11.5% of patients had died, 5.8% were on invasive mechanical ventilation, 22.9% remained in the hospital, and the remaining had been discharged from the hospital (Table 1). We used these outcomes in VIOLET to approximate Day 7 outcomes on the COVID Ordinal Outcome scale that we may observe in this trial.

Table 1. Patient status 14 days ("Day 15") after randomization in the VIOLET trial.[35]

Patient Status	Percentage of patients
Deceased	11.5%
Invasive mechanical ventilation	5.8%
Hospitalized, not on invasive mechanical ventilation	21.9%
Discharged from the hospital	60.8%

We plan to use a Bayesian analysis of the evolving data which allows flexibility in the number and timing of the interim analyses. If we determine there is >95% probability of the odds ratio being >1.0, the DSMB should consider stopping the trial for efficacy. If we determine there is >90% probability that the odds ratio is <1.1, the DSMB should consider stopping the trial for futility. If we determine there is >70% probability that the odds ratio is <0.70, the DSMB should consider stopping the trial for harm. We will use a prior odds ratio of 1.0 (equal chance of harm and benefit; mean log OR of 0.0) and a prior distribution of the standard error for its log set at 0.352 for tests of efficacy and a non-informative prior for tests of futility and harm. The results will be reported in a similar manner to those published by Goligher et al.[36] One advantage of Bayesian analysis is that stopping guidelines are not binding and the DSMB is charged with using judgement and data both internal and external to the trial to make any irrevocable decision.

If the trial enrolls 30 participants, further enrollment will be paused until the DSMB reviews data on the primary outcome from all enrolled participants; a decision to continue enrollment will be made by the DSMB while the investigators remain blinded.

Stopping points:

We will conduct a futility analysis at 20 patients. If no efficacy is observed or the primary endpoint indicates worse outcomes, the study will be halted.

7.2 Planned deviations from this design
This trial is being conducted in a rapidly evolving pandemic of a novel disease. Thus, we have developed a statistical plan with flexibility to be modified based on results from other concurrently conducted trials and emerging data on the clinical epidemiology of COVID-19. The primary advantage of a Bayesian monitoring plan is that whenever the trial is stopped the inference only depends on the data and not the original statistical plan that was developed at a time when less was known about COVID-19 and potentially effective treatments.

8. DATA QUALITY MONITORING AND STORAGE

8.1 Data Quality Monitoring
Data quality will be reviewed remotely using front-end range and logic checks at the time of data entry and back-end monitoring of data using application programming interface tools connecting the online database to statistical software to generate data reports. Patient records and case report forms will also be examined by site personnel for a randomly selected 5-10% sample to evaluate the accuracy and completeness of the data entered into the database and monitor for protocol compliance. The coordinating center will perform remote monitoring of each study site to examine the completeness and accuracy of informed consent documents for study participants, documentation of eligibility criteria, and the completeness of study outcome collection.

8.2 Data Storage
Data will be entered into a secure online database. All data will be maintained in the secure online database until the time of study publication. At the time of publication, a de-identified version of the database will be generated.

9. RISK ASSESSMENT

9.1 Potential Risk to Participants
Although both magnesium citrate and the probiotic Floranexs are FDA approved medications with an established safety profile, potential risks exist to participating in this study of magnesium citrate plus the probiotic Floranex versus placebo for the treatment of COVID-19. Mild hypermagnesemia (increases of >1meq) are typical because the majority of the medication remains intraluminal. Patients with renal insufficiency (<30cc/min) are at risk for greater increases in systemic magnesium levels and thus they will be excluded from study participation. Below, we outline side effects based on serum magnesium levels.

9.1.1 Potential risks of receiving magnesium citrate
Common Side effects of magnesium citrate include:
Abdominal cramping
Diarrhea
Electrolyte imbalance
Gas (flatulence)
Nausea
Vomiting
Hypermagnesemia (high levels of magnesium in the blood.

Side effects that can occur with Hypermagnesemia:
Nervous system
- Muscle weakness, decrease in tendon reflexes, mental confusion, or sedation when serum magnesium (Mg) levels increase to 4 to 7 mEq/L,
- Slowed respiratory rate and hypotension when serum magnesium (Mg) levels increase to 5 to 10 mEq/L

- Profound mental depression, areflexia, coma and respiratory paralysis when serum Mg levels increase to 10 to 15 mEq/L
- Death possible when serum Mg levels rise to 15 mEq/L or higher.

Cardiovascular
- Hypotension may be observed when serum Mg levels rise to 5 to 10 mEq/L.
- Hypotension, depressed myocardial conductivity, and bradyarrhythmias may be associated with levels greater than 10 mEq/L.
- Asystole when levels rise to 25 mEq/L.

Metabolic
- Hypocalcemia

Gastrointestinal
- Nausea when serum Mg levels rise to 4 to 5 mEq/L.
- Rare cases of paralytic ileus associated with serum Mg levels greater than 5 mEq/L have been reported.
-

9.1.2 Potential risks of receiving the probiotic Floranexs

Risks include is gas, bloating and diarrhea**.**

9.1.3 Potential risks of receiving placebo with COVID-19

One potential risk to participating in this study is receiving placebo rather than magnesium citrate plus the probiotic Floranex. This risk is only relevant if magnesium citrate plus a the probiotic Floranex is ultimately found to be an effective therapy for COVID-19 and is not relevant if magnesium citrate plus the probiotic Floranex is ultimately found to be an ineffective therapy for COVID-19. This trial protocol minimizes this risk through rigorous design to minimize the number of patients who must be enrolled to determine whether magnesium citrate plus the probiotic Floranex is an effective therapy for COVID-19, excluding patients who decline to participate because they feel their optimal care requires magnesium citrate plus the probiotic Floranex, excluding patients whose treating clinicians declines to allow enrollment because they feel the patient's optimal care requires treatment with magnesium citrate plus the probiotic Floranex, and specifying procedures for stopping the study drug, unblinding, and allowing open-label administration of magnesium citrate plus the probiotic Floranex for patients who experience clinical deterioration during the study period.

9.2 Minimization of Risk

Federal regulations at 45 CFR 46.111(a)(1) require that risks to participants are minimized by using procedures which are consistent with sound research design. This trial protocol incorporates numerous design elements to minimize risk to patients that meet this human subject protection requirement. Magnesium citrate has been approved by the Food and Drug Administration and has been used in clinical practice for decades in a number of patient populations with an established safety profile. The dose and route of administration of magnesium citrate and the probiotic Floranexs in this trial are comparable to the dose and route of administration approved for uses already approved by the FDA. The trial protocol includes on-study monitoring to minimize the risk to patients during therapy. This monitoring also includes both automated electronic health record and manual study personnel review for medications with potential interactions with magnesium citrate and the probiotic Floranexs. The trial protocol includes monitoring of adverse events, clinical outcomes, and interim analyses by an independent data and safety monitoring board empowered to stop or modify the trial at any time.

9.3 Potential Benefit
Study participants may or may not receive any direct benefits from their participation in this study. Administration of magnesium citrate plus the probiotic Floranex may improve clinical outcomes among adults hospitalized for COVID-19 infection.

9.4 Risk in Relation to Anticipated Benefit
Federal regulations at 45 CFR 46.111 (a)(2) require that "the risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result." Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits.

10. HUMAN SUBJECTS PROTECTIONS
Each study participant or a LAR must sign and date an informed consent form. Approval of the central institutional review board will be required before any participant is entered into the study.

10.1 Selection of Subjects
Federal regulations at 45 CFR 46(a)(3) require the equitable selection of subjects. The Vanderbilt University Medical Center emergency departments, hospital wards, and ICUs will be screened to determine if any patient meets inclusion and exclusion criteria. Data that have been collected as part of the routine clinical care of the patient will be reviewed to determine eligibility. If any patient meets criteria for study enrollment, then the attending physician responsible for his or her care will be asked for permission to approach the patient or his or her LAR for informed consent. Study exclusion criteria neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals for participation in the research. Hence, the recruitment of participants conforms to the principle of distributive justice.

10.2 Justification of Including Vulnerable Subjects
The present research aims to investigate the safety and efficacy of magnesium citrate plus the probiotic Floranex for the treatment of patients with COVID-19 who are at high risk for respiratory failure and mortality. Due to the nature of this patient population, many of these patients will have impaired decision-making capabilities. Moreover, those with intact decision-making capacities probably have milder disease than those with impaired capacity. Therefore, the validity of the study and its generalizability to severely ill patients would be compromised by enrolling only those participants with retained decision-making capacity. Hence, participants recruited for this trial are not being unfairly burdened with involvement in this research.

10.3 Informed Consent
Federal regulations 45 CFR 46.111(a)(5) require that informed consent will be sought from each patient or the patient's LAR. Study personnel obtaining informed consent are responsible for ensuring that the patient or LAR understands the risks and benefits of participating in the study, answering any questions the patient or LAR may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's or LAR's willingness to permit the patient's continued participation in the trial. The study personnel obtaining informed consent will make every effort to minimize coercion. All patients or their LARs will be informed of the objectives of the study and the potential risks. The informed consent document will be used to explain the risks and benefits of study participation to the patient or LAR in simple terms before the patient is entered into the study, and to confirm that the patient or LAR is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or LAR. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures including administration of study agent.
For additional details, see Section 3.

10.4 Continuing Consent

Patients for whom consent was initially obtained from a LAR, but who subsequently regain decision-making capacity while in hospital will be approached for consent for continuing participation, including continuance of data acquisition. The consent form signed by the LAR should reflect that such consent should be obtained. The process for obtaining consent from these patients will be the same as that outlined in section 3.

10.5 Withdrawal of Consent

Participating patients may withdraw or be withdrawn (by the LAR, treating physician, or investigator) from the trial at any time without prejudice. Data recorded up to the point of withdrawal will be included in the trial analysis, unless consent to use data has also been withdrawn. Withdrawal of consent prior to receipt of study drug will constitute a screen-failure and will be recorded. Withdrawal of consent after randomization and administration of one or more doses of study drug will lead to discontinuation of study interventions but site staff will request access to medical records for data related to the trial.

10.6 Identification of Legally Authorized Representatives

Many of the patients approached for participation in this research protocol will have impaired decision-making capacity due to critical illness and will not be able to provide informed consent. Accordingly, informed consent will be sought from the patient's LAR.

Regarding consent from the LAR, the existing federal research regulations ('the Common Rule') states at 45 CFR 46.116 that "no investigator may involve a human being as a subject in research…unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative"; and defines at 45 CFR 46 102 (c) a LAR as "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedures(s) involved in the research." The Office of Human Research Protections (OHRP) defined examples of "applicable law" as being state statutes, regulations, case law, or formal opinion of a State Attorney General that addresses the issue of surrogate consent to medical procedures. Such "applicable law" could then be considered as empowering the LAR to provide consent for participant participation in the research. Interpretation of "applicable law" may be state specific and will be addressed by the central IRB.

According to a previous President's Bioethics Committee (National Bioethics Advisory Committee (NBAC)), an investigator should accept a relative or friend of the potential participant who is recognized as an LAR for purposes of clinical decision making under the law of the state where the research takes place.[46] Finally, OHRP has stated in their determination letters that a surrogate could serve as a LAR for research decision making if such an individual is authorized under applicable state law to provide consent for the "procedures" involved in the research study

10.7 Justification of Surrogate Consent

According to the Belmont Report, respect for persons incorporates at least two ethical convictions; first, that individuals should be treated as autonomous agents, and second, that persons with diminished autonomy are entitled to protection. One method that serves to protect patients is restrictions on the participation of patients in research that presents greater than minimal risk. Commentators and research ethics commissions have held the view that it is permissible to include incapable participants in greater than minimal risk research as long as there is the potential for beneficial effects and that the research presents a balance of risks and expected direct benefits similar to that available in the clinical setting.[47] Several U.S. task forces have deemed it permissible to include incapable participants in research. For example, the American College of Physicians' document allows surrogates to consent to research involving incapable participants only "if the net additional risks of participation are not substantially greater than the risks of standard treatment".[48] Finally, NBAC stated that an IRB may approve a protocol that presents greater than minimal risk but offers the prospect of direct medical benefits to the participant, provided that "the potential subject's LAR gives permission…".[46]

Consistent with the above ethical sensibilities regarding the participation of decisionally incapable participant in research and the previous assessment of risks and benefits in the previous section, the present trial presents a balance of risks and potential direct benefits that is similar to that available in the clinical setting.

10.8 Additional Safeguards for Vulnerable Participants

The present research will involve participants who might be vulnerable to coercion or undue influence. As required in 45CFR46.111(b), we recommend that sites utilize additional safeguards to protect the rights and welfare of these participants. Such safeguards might include but are not limited to: a) assessment of the potential participant's capacity to provide informed consent, and b) the availability of the LAR to monitor the participant's subsequent participation and withdrawal from the study. The specific nature of the additional safeguards will be left to the discretion of the central IRB, in conjunction with the sites.

10.9 Confidentiality

Federal regulations at 45 CFR 46 111 (a) (7) requires that when appropriate, there are adequate provisions to protect the privacy of participants and to maintain the confidentiality of data. At no time during the course of this study, its analysis, or its publication will patient identities be revealed in any manner. The minimum necessary data containing patient or provider identities will be collected. All patients will be assigned a unique study ID number for tracking. All data collected for this study will be entered directly into a secure online database. All data will be maintained in the secure online database until the time of study publication. At the time of publication, a de-identified version of the database will be generated. Further, tools within the secure online database will be used so that only the coordinating center and investigators from the enrolling site will have access to data from participants enrolled at that site.

11. ADVERSE EVENTS

Assuring patient safety is an essential component of this protocol. Magnesium citrate has been approved by the Food and Drug Administration and used in clinical practice for decades with an established safety profile. Use of magnesium citrate plus the probiotic Floranex for the treatment of acute respiratory infection due to COVID-19, however, raises unique safety considerations. This protocol addresses these considerations through:

1. Exclusion criteria designed to prevent enrollment of patients likely to experience adverse events with receipt of magnesium citrate or the probiotic Floranexs, such as those with creatinine clearance of <30cc/min;
2. Proactive education of treating clinicians regarding medication interactions relevant to use of magnesium citrate plus the probiotic Floranex in the inpatient setting;
3. On-study monitoring of co-interventions (e.g., medications) and patient characteristics (e.g., EKG) to intervene before adverse events occur;
4. Systematic collection of safety outcomes relevant to use of magnesium citrate plus the probiotic Floranex in this setting;
5. Structured reporting of adverse events

11.1 Adverse Event Definitions

Adverse Event: Any untoward medical occurrence associated with the use of a drug or a study procedure, whether or not considered drug related.

Serious Adverse Event: A serious adverse event is any adverse event that results in one of the outcomes listed in section 11.3 below.

Adverse Reaction: An adverse reaction means any adverse event caused by a study intervention. An adverse reaction is a subset of all suspected adverse events where there is a reason to conclude that the study intervention caused the event.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the study procedures caused the adverse event. Reasonable possibility means there is evidence to suggest a causal

relationship between the study procedures and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction.
Suspected Unexpected Serious Adverse Reaction (SUSAR): An adverse reaction that is both unexpected (not consistent with risks outlined in the study protocol or investigator brochure), serious, and meets the definition of a suspected adverse reaction.

11.2 Safety Monitoring

Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The Investigators will determine daily if any adverse events occur during the period from enrollment through **study day 7** or hospital discharge, whichever occurs first and will determine if such adverse events are reportable. Thereafter, adverse events are not required to be reported unless the investigator feels the adverse event was related to study drug or study procedures.
The following adverse events will be considered reportable and thus collected in the adverse event case report forms:

- Serious adverse events
- Non-serious adverse events that are considered by the investigator to be related to study procedures or of uncertain relationship (Appendix C)
- Events leading to permanent discontinuation of study drug

Study-specific clinical outcomes (Primary, Secondary and Safety Outcomes and Assessments During the Study), including serious outcomes such as organ failures and death, are systematically recorded in the case report forms and are exempt from adverse event reporting unless the investigator deems the event to be related to the administration of study drug or the conduct of study procedures (or of uncertain relationship) as outlined in Appendix C.

After randomization, adverse events must be evaluated by the investigator. If the adverse event is judged to be reportable, as outlined above, then the investigator will report to the Clinical Coordinating Center (CCC) their assessment of the potential relatedness of each adverse event to the study drug or protocol procedure via electronic data entry. Investigators will assess if there is a reasonable possibility that the study procedure caused the event, based on the criteria outlined in Appendix C. Investigators will also consider if the event is unexpected. Unexpected adverse events are events not listed in the study protocol and the package insert for the study drugs will also determine if adverse events are unanticipated given the patient's clinical course, previous medical conditions, and concomitant medications.
If a patient's treatment is discontinued as a result of an adverse event, study site personnel must also report the circumstances and data leading to discontinuation of treatment in the adverse event case report forms.

11.3 Serious Adverse Events

Serious adverse event collection begins after randomization and study procedures have been initiated. If a patient experiences a serious adverse event after consent, but prior to randomization or starting study procedures, the event will NOT be collected. Study site personnel must alert the CCC of any **serious and study procedure related** adverse event within 24 hours of investigator awareness of the event. Alerts issued via telephone are to be immediately followed with official notification on the adverse event case report form. See Appendix C for reporting timelines for serious, unexpected, study related events (SAEs) and serious, unexpected suspected adverse reactions (SUSARs)
As per the FDA and NIH definitions, a serious adverse event is any adverse event that results in one of the following outcomes:

- Death
- A life-threatening experience (that is, immediate risk of dying)
- Prolonged inpatient hospitalization or re-hospitalization

As per http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm: Report if admission to the hospital or prolongation of hospitalization was a result of the adverse event. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).

- Persistent or significant disability/incapacity
  As per http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm: Report if the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.

Reportable serious adverse events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious adverse events will be collected until hospital discharge, regardless of the investigator's opinion of causation.

12. DATA AND SAFETY MONITORING BOARD (DSMB)

The principal role of the DSMB is to assure the safety of participants in the trial. They will regularly monitor data from this trial, review and assess the performance of its operations, and make recommendations to the steering committee with respect to:

- Review of adverse events
- Interim results of the study for evidence of efficacy or adverse events
- Possible early termination of the trial because of new external information, early attainment of study objectives, safety concerns, or inadequate performance
- Possible modifications in the clinical trial protocol
- Performance of individual centers

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APPENDICES

Appendix A. Schedule of Events

Study Activity	Pre-enrollment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8 through Discharge	Day 29	Acute Care F/U (by phone if discharged prior to Day 7 or Day 29)	3 Mos. F/U
Eligibility assessment	X											
Pregnancy test (if applicable)	X											
Informed consent	X											
COVID Ordinal Outcome Score Assessment[1]	X		X	X	X	X	X	X	X	X	X	
Randomization		X										
Baseline Variable Data Collection		X										
Blood Sample Collection		X						X				
Nasal Swab Collection		X		X						X		
Sequential Organ Failure Assessment (SOFA)		X		X								
ARDS Evaluation[1]		X	X	X	X	X	X	X	X	X		
Study Drug Delivery		X										
Study Variable Data Collection[1]			X	X	X	X	X	X	X	X		
Assessment of Study Drug Adherence[1]		X	X	X	X	X	X					
Adverse Events Monitoring/Assessment		X	X	X	X	X	X	X		X	X	
Safety Outcomes Assessment		X	X	X	X	X	X	X	X	X		
Mortality Assessment								X		X	X	
Long-term Outcomes[2]												X

[1]Assessed if patient remains hospitalized; assessed by telephone follow-up if participant has been discharged
[2]Assessed in-person, by telephone or videophone.

Appendix B. Potential medication interactions with magnesium citrate

Magnesium containing drugs can potentially lower drug levels of some medications. We will advise treating physicians to allow there be a 6-hour window between administration of these drugs and magnesium citrate. This should not restrict the enrollment of someone taking these drugs. Here is a list of drugs that can be affected by magnesium citrate:

Demeclocycline
Dolutegravir
Doxycycline
Eltrombopag
Lymecycline
Minocycline
Oxytetracycline
Potassium Phosphates, Intravenously
Tetracycline
Ciprofloxacin
Deflazacort
Fleroxacin
Gemifloxacin
Levofloxacin
Moxifloxacin
Norfloxacin
Ofloxacin
Penicillamine
Sodium Phosphates, Intravenously
Vitamin D

Appendix C: Adverse Event Reporting and Unanticipated Events

As noted in section 11, investigators will report all "serious adverse events," defined as adverse events that are serious and have a reasonable possibility that the event was due to a study drug or procedure (or of uncertain relatedness), to the CCC within 24 hours. The CCC will then notify the NHLBI and Central Institutional Review Board (cIRB).

The Medical Monitor at the CCC will work collaboratively with the reporting investigator to determine if a serious adverse event has a reasonable possibility of having been caused by the study drug or study procedure, as outlined in 21 CFR 312.32**(a)**(1), and below. The Medical Monitor will be unblinded and will also determine if the event is unexpected for magnesium citrate or the probiotic Floranexs. An adverse is considered "unexpected" if it is not listed in the investigator brochure or the study protocol (21 CFR 312.32(a)). If a determination is made that a serious adverse event has a reasonable possibility of having been caused by a study procedure or the study drug, it will be classified as a suspected adverse reaction. If the suspected adverse reaction is unexpected, it will be classified as a serious unexpected suspected adverse reaction (SUSAR).

The CCC will report all unexpected deaths, serious and treatment related adverse events, and SUSARs to the DSMB, NHLBI, and cIRB within 7 days after receipt of the report from a clinical site. A written report will be sent to the NHLBI, DSMB, FDA, and the cIRB within 15 calendar days. The DSMB will also review all reported adverse events and clinical outcomes during scheduled interim analyses. The CCC will distribute the written summary of the DSMB's periodic review of reported adverse events to the cIRB in accordance with NIH guidelines (http://grants.nih.gov/grants/guide/notice-files/not99-107.html). The Medical Monitor will provide to Sandoz Pharmacovigilance any significant safety findings (without disclosing protected health information) during the conduct of the trial.

C.1. Unanticipated Problems (UP)

Investigators must also report Unanticipated Problems, regardless of severity, associated with study procedures within 24 hours. An unanticipated problem is defined as follows: any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
- Related or possibly related to participation in the research, in this guidance document, 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;
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

C.2. Determining Relationship of Adverse Events to Study Drug or Study Procedures

Investigators will be asked to grade the strength of the relationship of an adverse event to study drug or study procedures as follows:

- Definitely Related: The event follows: a) A reasonable, temporal sequence from a study procedure; and b) Cannot be explained by the known characteristics of the patient's clinical state or other therapies; and c) Evaluation of the patient's clinical state indicates to the investigator that the experience is definitely related to study procedures.
- Probably or Possibly Related: The event should be assessed following the same criteria for "Definitely Associated". If in the investigator's opinion at least one or more of the criteria are not present, then "probably" or "possibly" associated should be selected.

- Probably Not Related: The event occurred while the patient was on the study but can reasonably be explained by the known characteristics of the patient's clinical state or other therapies.
- Definitely Not Related: The event is definitely produced by the patient's clinical state or by other modes of therapy administered to the patient.
- Uncertain Relationship: The event does not meet any of the criteria previously outlined.

C.3. Clinical Outcomes that may be Exempt from Adverse Event Reporting

Study-specific outcomes of acute respiratory infection, COVID-19, and critical illness will be systematically collected for all patients in both study group and are exempt from adverse event reporting unless the investigator considers the event to be Definitely or Possibly Related (or of an Uncertain Relationship) to the study drug or study procedures. Examples of study-specific clinical outcomes include:

- Death not related to the study procedures
- Neurological events
    - Seizure
- Cardiovascular events
    - Receipt of vasopressors
    - Atrial or ventricular arrhythmia
    - Cardiac arrest
- Respiratory events
    - Hypoxemia requiring supplemental oxygen
    - Acute respiratory distress syndrome
    - Receipt of mechanical ventilation
    - Receipt of extra-corporeal membrane oxygenation
- Gastrointestinal events
    - Elevation of aspartate aminotransferase or alanine aminotransferase
    - Acute pancreatitis
- Renal events
    - Acute kidney injury
    - Receipt of renal replacement therapy
- Endocrine events
    - Symptomatic hypoglycemia
- Hematologic or coagulation events
    - Neutropenia, lymphopenia, anemia, or thrombocytopenia
- Dermatologic events
    - Severe dermatologic reaction (e.g., Steven's Johnson Syndrome)

Note: A study-specific clinical outcome may also qualify as a reportable adverse event. For example, a ventricular arrhythmia that the investigator considers Definitely or Possibly Related to the study drug would be both recorded as a study-specific clinical outcome and reported as a Serious and Definitely or Possibly Related Adverse Event.

C.4. Decision tree for determining if an adverse event is reportable

Figure 1. Decision tree for determining if an adverse event is reportable

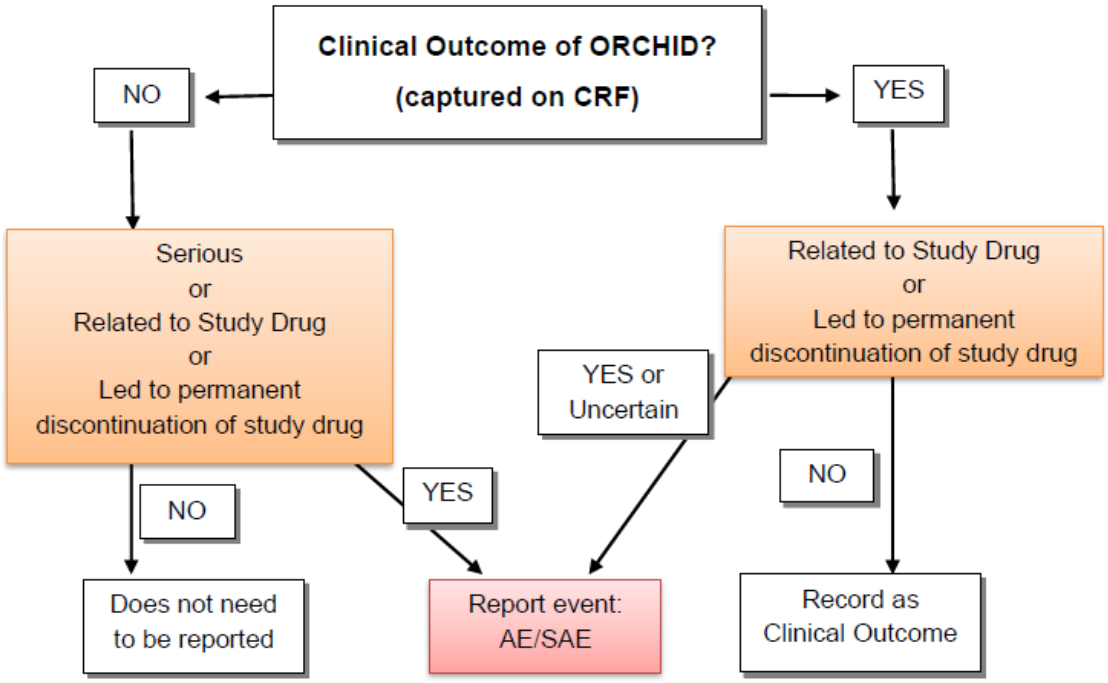

Figure adapted from Ranieri VM, Thompson BT, Barie PS et al. Drotrecogin alfa (activated) in adults with septic shock. N Engl J Med. 2012 May 31;366 (22):2055-64. PMID: 22616830.

Appendix D. Public Readiness and Emergency Preparedness Act

This study is being conducted to determine whether magnesium citrate plus a the probiotic Floranex can safely and effectively be used to mitigate, treat, or cure COVID-19 or limit the harm of the COVID-19 pandemic in accordance with the Secretary of the Department of Health and Human Services' (HHS's) Declaration under the Public Readiness and Emergency Preparedness Act for medical countermeasures against COVID-19 (COVID-19 Declaration) effective February 4, 2020. The purpose of this study is to test if magnesium citrate plus a the probiotic Floranex results in clinical benefit in patients hospitalized with COVID- 19.

Magnesium citrate has been approved by the FDA for other uses and its investigational use for COVID-19 in this study has been exempted by the FDA from investigational new drug application requirements pursuant to 21 CFR 312.2(b)(1). This study is conducted under a Research Project Cooperative Agreement with the National Heart, Lung, and Blood Institute.

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Appendix E

COVID Ordinal Outcome Scale

Patient State	Descriptor	Score
Uninfected	Uninfected, no viral RNA detected	0
Ambulatory Mild Disease	Asymptomatic, viral RNA detected	1
	Symptomatic, independent	2
	Symptomatic, assistance needed	3
Hospitalized Moderate Disease	Hospitalized, no oxygen needed	4
	Hospitalized, oxygen by mask or nasal prongs	5
Hospitalized Severe Disease	Hospitalized, oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation, pO_2/FiO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation, pO_2/FiO_2 <150 and vasopressors, dialysis or ECMO	9
Dead	Dead	10