RECOVER-VITAL: A Platform Protocol for Evaluation of Interventions for Viral Persistence, Viral Reactivation, and Immune Dysregulation in Post-Acute Sequelae of SARS-CoV-2 Infection (PASC)

National Clinical Trial (NCT) Identified Numbers: NCT05595369, NCT05965726

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Funded by: National Institutes of Health

Version Number: v3.0

18-MAY-2023

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STATEMENT OF COMPLIANCE

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

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

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

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

1.1 SYNOPSIS

Title:	RECOVER-VITAL: A Platform Protocol for Evaluation of Interventions for Viral Persistence, Viral Reactivation, and Immune Dysregulation in Post-Acute Sequelae of SARS-CoV-2 Infection (PASC)
Study Description:	This study is a platform protocol designed to be flexible so that it is suitable for a wide range of settings within health care systems and community settings where it can be integrated into COVID-19 programs and subsequent treatment plans. This protocol describes a prospective, multi-center, multi-arm, double-blind, randomized, controlled, platform trial, with different interventions organized as appendices to the protocol. Each appendix evaluates potential mechanisms of action, efficacy, and safety of antivirals and other therapeutics in individuals with PASC, according to the platform protocol objectives. The hypothesis is that persistent viral infection and/or overactive/chronic immune response and inflammation are underlying contributors to PASC and that antiviral and other applicable therapies may result in viral clearance or decreased inflammation and improvement in PASC symptoms.
Objectives:	Primary: Evaluate the effect of intervention(s) versus control on symptom-specific patient-reported outcome measures within each PASC symptom cluster at Day 90 Secondary: 1. Evaluate the effect of intervention(s) versus control on performance-based outcomes within each PASC symptom cluster

	2. Characterize the safety and tolerability of intervention(s) in PASC Exploratory: 1. Investigate mechanisms of potential intervention (s) efficacy 2. Compare the effect of intervention(s) on symptom burden and health related quality of life (HRQOL) versus control 3. Evaluate the durability of intervention(s) response 4. Evaluate intervention(s) effect on healthcare utilization 5. Describe the effect of intervention(s) versus control across exploratory performance-based outcomes and patient reported outcomes (PROs) within each PASC symptom cluster 6. Determine biomarkers associated with PASC symptom clusters and intervention-associated recovery
Study Population:	Each study intervention appendix will require approximately 900 evaluable adult participants who experience at least one of three predominant symptom clusters: cognitive dysfunction, autonomic dysfunction, or exercise intolerance. Overall enrollment for the platform protocol will depend on the number of screen failures, the number of study intervention appendices that are added, the ability to pool their control groups for analysis, and adjustments to sample size based on study data. An estimated sample size of approximately 900 evaluable participants per study intervention appendix is expected to be sufficient to provide adequate proof-of-concept that will enable optimal design and facilitate rapid transition to phase 3 trials for interventions that demonstrate preliminary evidence of benefit. The goal is to have a diverse population, including underserved communities and racial/ethnic populations frequently underrepresented in clinical research.
Phase:	Appendices including phase 2 and phase 3 studies
Description of Sites/Facilities Enrolling Participants:	Participants may be recruited from acute COVID-19 trials and existing RECOVER initiatives including, but not limited to, the longitudinal cohort, as well as research sites and communities. Approximately 100 sites in the US will participate.
Description of Study Intervention:	Each study intervention appendix describes a different study drug/drug combination/duration and control. The following arms will be included in each study appendix: • Study Drug Arm: study drug(s) of interest (intervention(s), see appendices) • Control Arm: control
Participant Duration:	The duration of dosing will depend on the intervention. Participants will be in the study for approximately 6 months from the time of initiating study drug administration until their end of study visit.

1.2 SCHEMA FOR PHASE 2 STUDY DESIGNS

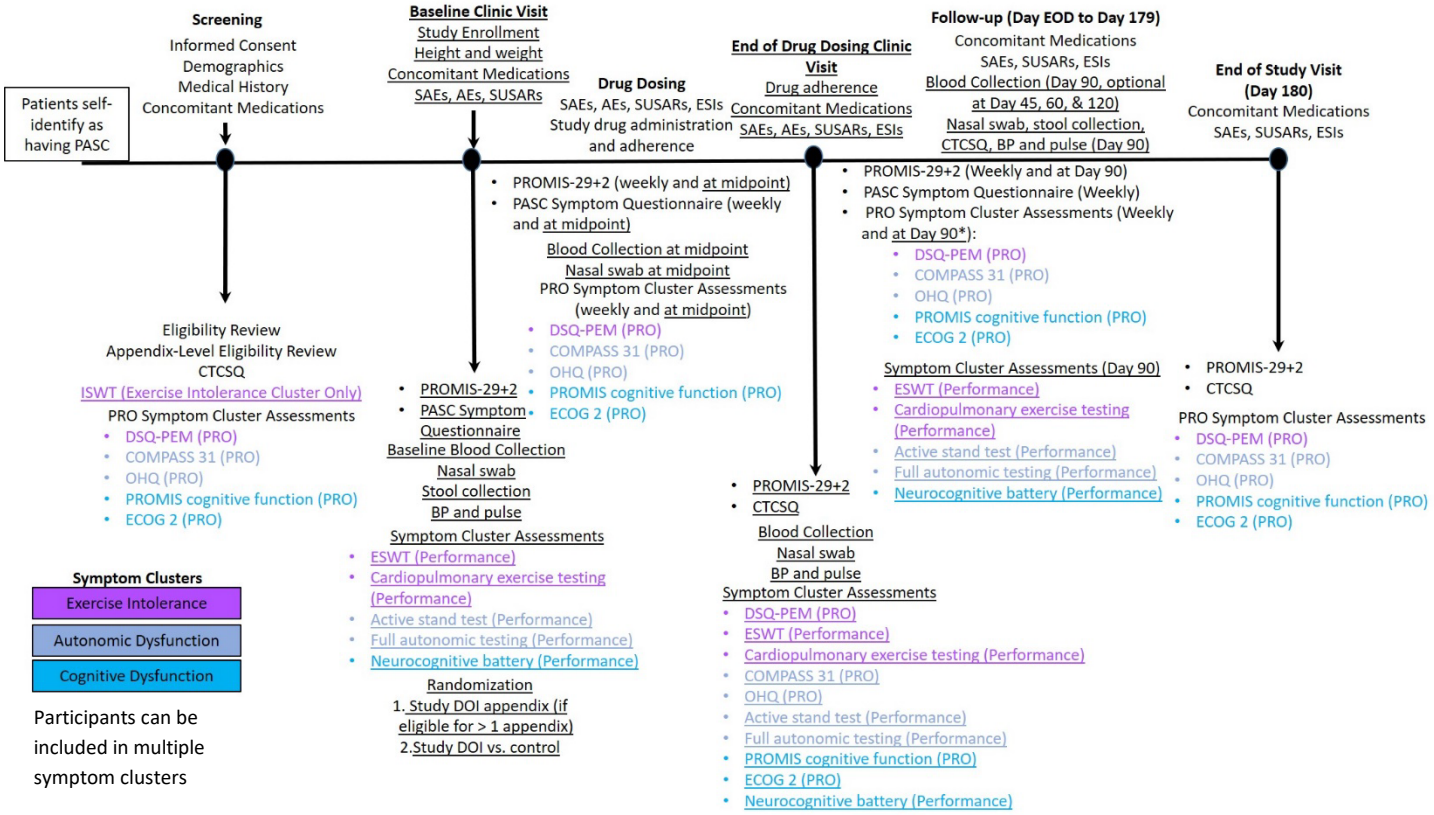

Figure 1. Study schema for phase 2 study designs

Underline in Figure 1 indicates an in-person visit; *Day 90 primary outcome assessment

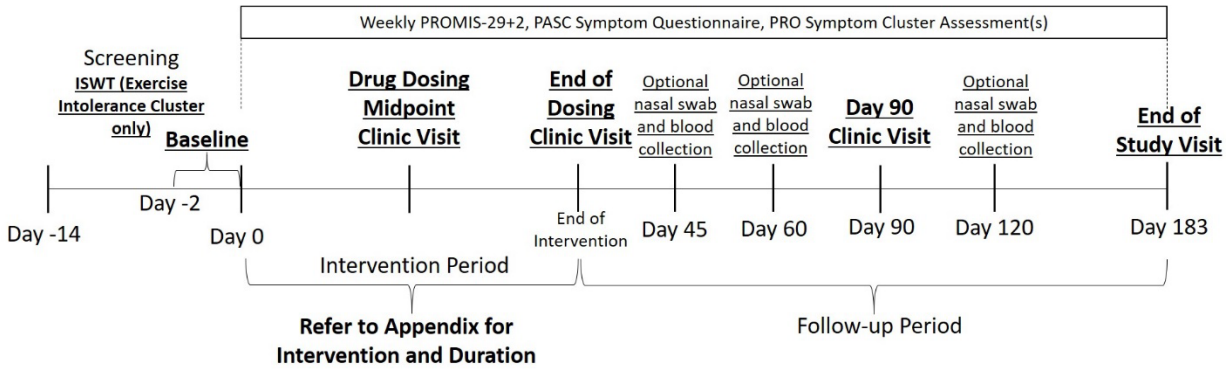

Figure 2. Concise study schema

Underline in Figure 2 indicates and in-person visit where study outcomes and other procedures will be assessed.

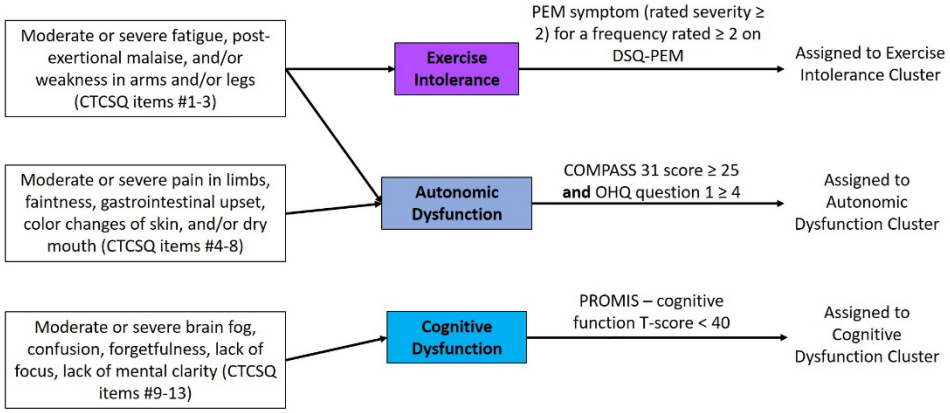

Figure 3. Symptom cluster assignment flow.

Participants who report having at least two moderate symptoms from the same symptom cluster or one severe symptom from the Cluster Targeted COVID-19 Symptom Questions (CTCSQ) will be screened for symptom clusters based on which symptoms are reported as moderate or severe. Arrows from symptom boxes to cluster boxes indicate which symptom clusters should be assessed based on the moderate or severe symptoms. Arrows from cluster boxes to assignment designation indicate the required scoring of the PRO in order to be assigned to the cluster. Participants may be screened for and assigned to multiple symptom clusters.

1.3 KEY ROLES

Platform Protocol Co-Chair/IND Sponsor: Kanecia Zimmerman, MD, PhD	Appendix A Principal Investigator: Lindsey Baden, MD
Platform Protocol Co-Chair: Richard Whitley, MD	

2 INTRODUCTION

2.1 STUDY RATIONALE

Post-acute sequelae of SARS-CoV-2 infection (PASC), also known as Long COVID, is a chronic condition present in up to 80% of SARS-CoV-2-infected, hospitalized patients and 40 to 70% of non-hospitalized patients with COVID-19.[1-4] Symptoms of PASC can be debilitating. The personal and global impact of these long-term symptoms from SARS-CoV-2 infection can be devastating, and the number of Long COVID patients is escalating. With the increasing number of people infected with SARS-CoV-2, an urgent and unmet clinical need exists to better understand the pathophysiology of PASC and to develop targeted therapeutics to resolve the disease more rapidly and restore patients' health. This study aims to investigate various interventions including antivirals, immunomodulatory agents, and other therapeutics or combinations thereof, and evaluate their use in treating PASC, including 1) mechanisms of potential efficacy, 2) safety and tolerability, 3) optimal outcomes and endpoints, and 4) preliminary efficacy. If successful, this trial will rapidly provide the foundational evidence for future phase 3 studies to further establish intervention efficacy in the PASC population.

2.2 BACKGROUND

In 2019, a novel coronavirus-disease (COVID-19) emerged in Wuhan, China. A month later the Chinese Center for Disease Control and Prevention identified a new beta-coronavirus (SARS coronavirus 2, or SARS-CoV-2) as the etiological agent.[5] The clinical manifestations of COVID-19 range from asymptomatic infection or mild, transient symptoms to severe viral pneumonia with respiratory failure.

COVID-19 has led to the death of more than 6 million people worldwide; however, this disease has affected even more lives through often-debilitating symptoms lingering long after acute SARS-CoV-2 infection. Post-acute sequelae of SARS-CoV-2 infection, also known as PASC, is a chronic condition that affects nearly every organ system, with more than 200 individual symptoms, ranging from new-onset anxiety, depression, and cognitive difficulties to shortness of breath, dizziness, and arrhythmias.[6] Moreover, PASC can occur regardless of severity of acute COVID-19 disease, and it impacts across socioeconomic, racial and ethnic, and age strata. These prolonged symptoms open the door for substantial short- and long-term individual and societal costs, including healthcare costs and inability to work. Prolonged symptoms have kept individuals out of work, which has exacerbated poverty in the underserved, historically minoritized populations and worsened a decades-long mental health crisis. Considering these costs, identification of safe and effective methods to treat and prevent the occurrence of PASC represents an urgent, unmet public health need.

To address this need, the NIH has launched the RECOVER initiative across the nation (RECOVER: Researching COVID to Enhance Recovery) to better understand the disease. The RECOVER Initiative brings together patients, caregivers, clinicians, community leaders, and scientists from across the nation to understand, prevent, and treat PASC. The RECOVER Consortium represents and supports researchers

who are leading studies on PASC at more than 200 sites around the country. These studies have a diverse group of participants, including adults, pregnant people, and children. Data from the RECOVER initiative, as well as existing literature, have highlighted three predominant symptom clusters, including exercise intolerance with post-exertional malaise (PEM), cognitive dysfunction, and autonomic dysfunction, which are frequently reported and of substantial importance to patients. In addition, data have revealed substantial heterogeneity in symptomology and presentation, even among those within a specific symptom cluster, and in many cases the lack of concordance between objective findings and reported symptoms.[6,7]

Emerging research is also beginning to identify risk factors for PASC. Women are disproportionately impacted by PASC relative to men at an approximately 3:1 ratio, with this sex difference appearing to fade in older individuals 60 to 70 years of age.[8-10] Longitudinal, multi-omic profiling of COVID patients has revealed several anticipating risk factors for PASC at the time of diagnosis, including type 2 diabetes, SARS-CoV-2 RNAemia, Epstein-Barr Virus viremia, and autoantibodies.[11]

Three etiologies have emerged as leading candidates for instigating the manifestations of PASC: disordered anticoagulation, immune dysregulation, and viral persistence/reactivation.[12,13] In particular, prior investigators have demonstrated in preliminary data that SARS-CoV-2 can persist throughout the body for up to 230 days after acute infection[14], and that viral RNA has been identified within the gut mucosa and plasma of the majority of patients with PASC, but not within those without PASC.[15] Direct targeting of viral persistence, coagulation, and immune dysregulation with therapeutic agents warrants further investigation.

Preliminary data suggest that antiviral treatment of those with lingering symptoms of COVID-19 beyond four weeks after acute infection can result in symptom improvement. In a cohort study of 29 adults with lingering symptoms ranging from fatigue to inappropriate tachycardia, and many with severely reduced functional status (48%) on a patient reported scale, 18 patients received antiviral therapy (lopinavir/ritonavir and hydroxychloroquine). A statistically significant improvement in functional status, defined as at least a one-point improvement on the functional scale, was observed at some point over a 6-week period among those receiving treatment compared to those who did not. Half of the patients who received antiviral therapy had SARS-CoV-2 RNA detected at treatment initiation via RT-PCR in plasma; no SARS-CoV-2 was detected in the plasma of these 9 patients during the 1st week following treatment.[16] While these data are promising, the heterogeneity and novelty of PASC, discordance between reported symptoms and objective findings,[6,7,17] and lack of validated measures and endpoints specific to this disease process highlight the importance of studying antiviral and other therapies' effects in the context of well-controlled clinical trials to ensure that optimal and appropriate therapies are made rapidly available for patients. The proposed clinical trials will evaluate potential mechanisms underpinning PASC and PASC improvement and test the utility of endpoints and outcome measures that have been well-established in other disease processes in the PASC population. In addition, collection of key biospecimens can assist in the development of biomarkers associated with PASC symptom clusters and response to therapy allowing a better understanding of pathogenesis and potentially targeted interventions. Phase 3 appendix trials will be sized to build on learnings from phase 2 trials to more definitively define drug efficacy for interventions that show promise in preliminary evaluations.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Potential risks of this study include those associated with the specific study drug (refer to appendices for details), blood draws, nasal swabs, performance measures, and loss of confidentiality.

Risks associated with blood draws include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although unlikely.

Risks associated with nasal swabs include mild irritation, insignificant local pain, and minor bleeding.

Risks associated with the incremental and endurance shuttle walk tests (ISWT/ESWT) include tiredness, shortness of breath, or palpitations during or after the test. The active stand test and full autonomic testing may cause some participants to faint or feel lightheaded or weak. The cardiopulmonary exercise test may result in fatigue, shortness of breath, possible bronchospasm, cardiac arrhythmia, and/or syncope.

There is also a risk of loss of confidentiality. However, coding all participant data with a unique identification number will minimize risk to loss of participant confidentiality.

2.3.2 KNOWN POTENTIAL BENEFITS

Participants may benefit from the potential clearance of the viral infection or reduction in inflammation and improvement in PASC symptoms.

Society in general and future patients infected with SARS-CoV-2 may benefit from the study's results, which aim to provide a better understanding of the benefit/risk of antiviral or anti-inflammatory treatment for PASC and alleviation of PASC symptoms.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Potential benefits may outweigh potential risks. Post-acute sequelae of SARS-CoV-2 infection (PASC) is a significant health issue and can have consequences that impact quality of life. These consequences are extremely important for the individual and also impact the society at large.

3 OBJECTIVES AND ENDPOINTS

The objectives, outcome measures, and endpoints for the study are listed in Table 1, below. Further details on outcome measures and endpoints are provided in Section 10 and the Statistical Analysis Plan (SAP).

Table 1. Summary of study objectives, endpoints, and outcome measures.

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Primary		
Evaluate the effect of intervention(s) versus control on symptom-specific patient-reported outcome measures within each PASC symptom cluster at Day 90	**Cognitive dysfunction symptom cluster:** PROMIS – cognitive 8a **Autonomic dysfunction symptom cluster:** Orthostatic Hypotension Questionnaire (OHQ) **Exercise intolerance symptom cluster:** Modified DePaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM)	Primary endpoint: Meeting pre-specified change from baseline to Day 90 on cluster-specific PRO outcome (yes/no) Exploratory endpoint: PRO outcome at Day 90 See Section 10.5.1
Secondary		
Evaluate the effect of intervention(s) versus control on performance-based outcomes within each PASC symptom cluster	**Cognitive dysfunction symptom cluster:** neurocognitive battery **Autonomic dysfunction symptom cluster:** active stand test **Exercise intolerance symptom cluster:** endurance shuttle walk test (ESWT)	Primary endpoint: Meeting pre-specified change from baseline to Day 90 on cluster-specific performance outcome (yes/no) Exploratory endpoint: Performance outcome at Day 90 See Section 10.5.2
Characterize the safety and tolerability of intervention(s) in PASC	• Serious adverse events (SAEs) • Events of Special Interest (ESIs) • Adverse events (AEs) • Adherence in intervention versus control	• Occurrence of individual SAEs • Occurrence of any one or more SAE • Occurrence of AEs and SAEs leading to discontinuation • Occurrence and duration of ESIs • Number of missed doses (adherence) See Section 10.5.3
Exploratory		
Investigate mechanisms of potential intervention(s) efficacy	• Antigenemia (plasma spike protein antigen and other markers of viral persistence and reactivation)	Change from baseline through end of study / final follow-up of the following:

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
	• Viral RNA in nasal swabs • Blood inflammatory markers • Coagulation markers • Endogenous and exogenous metabolites	• Viral clearance, persistence, and recurrence • Immune dysfunction • Coagulation • Biologic system functioning See Section 10.5.4
Evaluate intervention(s) effect on healthcare utilization	Healthcare utilization, including medical visits and hospitalizations	• Cumulative medical visits in intervention vs control • Cumulative hospitalizations in intervention vs control See Section 10.5.4
Describe the effect and durability of intervention(s) versus control across performance-based outcomes and patient reported outcomes (PROs) within each PASC symptom cluster	**All symptom clusters:** • PASC Symptom Questionnaire • PROMIS-29+2 • Global impression of change **Cognitive dysfunction symptom cluster:** • PROMIS - cognitive 8a • ECOG 2 **Autonomic dysfunction symptom cluster:** • COMPASS 31 • OHQ • Full autonomic testing (select sites) **Exercise intolerance symptom cluster:** • DSQ-PEM • Cardiopulmonary exercise test (select sites)	Outcome at Day 90 and End of Study See Section 10.5.4
Determine biomarkers associated with PASC symptom clusters and intervention-associated recovery	Outcome measures may include: • Active viral replication • Cytokine levels • Immune profiling (B and T cell) • Sanctuary site assessment • Infecting SARS-CoV-2 variant, impact SARS-CoV-2 immune state at time of infection	Change in biomarker levels correlated with clinical outcomes by PASC symptom cluster See Section 10.5.4

4 STUDY DESIGN

4.1 OVERALL DESIGN

This multi-center, multi-arm, master platform clinical trial protocol describes the overall design for prospective, double-blind, randomized, controlled trials that are described in appendices evaluating treatment of PASC in adults previously infected with SARS-CoV-2. Each appendix describes a sub-study that will evaluate interventions for PASC. The interventions may include different dosing durations or drug combinations for phase 2 or 3 investigation. Phase 2 appendices are designed to understand mechanisms of potential efficacy, safety and tolerability, and preliminary efficacy. Phase 3 appendices build upon the knowledge gained in phase 2 to more definitively evaluate the efficacy of interventions that show promise in Phase 2 trials. To decrease sample size, control participants may be pooled across appendices when appropriate. Rules for pooling data will be specified for each sub-study.

Within each sub-study, participants will be eligible to participate in assessments for up to three symptom clusters: cognitive dysfunction, autonomic dysfunction, exercise intolerance with PEM. Multiple symptom clusters are noted by up to 60% of individuals (unpublished data). Study intervention appendices may be added or removed according to adaptive design and/or emerging evidence; refer to the protocol appendices for further information on each intervention.

All participants will undergo blood draws to assess presence of virus and other biomarkers and report overall global health status and symptomatology as well as undergo organ-specific assessments, including PROs of functional status and objective assessments. Follow-up will be a combination of face-to-face visits when necessary to obtain objective clinical assessments and phone calls to be mindful of the participant burden.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Each intervention under this platform protocol will follow a double-blind, randomized trial design to compare one or more dose levels of the study intervention to control. Antiviral agents and other therapeutics of interest will be used. Additional interventions can be studied as new agents are available or as the understanding of the viral sanctuary sites and/or immune/inflammatory response is improved and favorable drug properties are discovered. Both phase 2 and phase 3 appendices will be incorporated into this platform to enable a more seamless transition from phase 2 to phase 3 trials for promising interventions by allowing phase 3 trials to take advantage of the phase 2 infrastructure for trial implementation. In some cases, incorporation of phase 2 and phase 3 appendices within a single protocol will also enable shared utilization of data across appendices.

Phase 2 and 3 trials conducted under this platform master protocol will share major design elements such as inclusion criteria, dose levels, follow-up schedule, and study outcomes. Because phase 3 designs are expected to be refined based on phase 2 experience, some implementation differences may occur, for example:

- **Inclusion criteria.** One or more symptom clusters may be dropped from phase 3 if phase 2 data suggest a lack of efficacy, insufficient volumes of eligible patients, or other factors that might impact a successful phase 3 outcome. Target populations may be narrowed to specific

subgroups if exploratory phase 2 data reveal participant factors that strongly predict presence or absence of treatment benefit.

- **Dose levels.** In general, each phase 3 study is expected to focus on a single intervention dose level. Phase 2 exploratory analyses will inform the selection of an optimal dose for phase 3 evaluation.
- **Follow-up schedule.** The duration and frequency of patient follow-up may be modified in light of phase 2 data. Factors to be considered include participant burden, patterns of missing data and drop out, fluctuations in symptoms over time, and uncertainty regarding the durability of a hypothesized treatment benefit.
- **Study outcomes.** An outcome designated as secondary in the current protocol may be elevated to primary in phase 3 if phase 2 data suggest that it outperforms a current primary outcome as a meaningful and sensitive measure of treatment benefit.

4.3 JUSTIFICATION FOR DOSE

The dose and duration of the intervention(s) will be based on the specific agent. Refer to the appendices for details.

4.4 END OF STUDY DEFINITION

The End of Study will occur when all participants have completed their End of Study Visit.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

1. ≥ 18 years of age at the time of enrollment
2. Previous suspected, probable, or confirmed SARS-CoV-2 infection, as defined by the Pan American Health Organization[18ϕ]

   ϕ Suspected and probable cases will only be allowed if they occurred before May 1, 2021, and will be limited to 10% of the study population. Otherwise, confirmed cases are required. Refer to the MOP for details.

   Suspected case of SARS-CoV-2 infection - Three options, A through C:

   A. *A person who meets the clinical OR epidemiological criteria. Clinical criteria: Acute onset of fever AND cough (influenza-like illness) OR Acute onset of ANY THREE OR MORE of the following signs or symptoms: fever, cough, general, weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, nausea, diarrhea, anorexia. Epidemiological criteria: Contact of a probable or confirmed case or linked to a COVID-19 cluster; or*
   B. *Acute respiratory infection with history of fever or measured fever of ≥ 38°C; and cough; with onset within the last 10 days; and who requires hospitalization); or*
   C. *With no clinical signs or symptoms, NOR meeting epidemiologic criteria with a positive professional use or self-test SARS-CoV-2 antigen-Rapid Diagnostic Test.*

Probable case of SARS-CoV-2 infection:

A. A patient who meets clinical criteria above AND is a contact of a probable or confirmed case or is linked to a COVID-19 cluster.

Confirmed case of SARS-CoV-2 infection - Two options, A through B:

A. A person with a positive nucleic acid amplification test, regardless of clinical criteria OR epidemiological criteria; or

B. Meeting clinical criteria AND/OR epidemiological criteria (See suspect case A). With a positive professional use or self-test SARS-CoV-2 Antigen-Rapid Diagnostic Test.

3. At least two moderate symptoms from the same Symptom Cluster or one severe cluster-associated symptom identified via the Cluster Targeted COVID-19 Symptom Questions (CTCSQ), see Section 8.10.3, with participant identifying new symptoms since COVID-19 illness and having persisted for at least 12 weeks[19]
4. Meeting PRO Symptom Cluster criteria for at least one Symptom Cluster (See Section 8.10.4)
5. Willing and able to provide informed consent, complete the surveys, clinical assessments, and return for all of the necessary follow-up visits

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study. Refer to appendices for additional appendix-level criteria:

1. Known active acute SARS-CoV-2 infection ≤ 4 weeks from consent
2. Known severe anemia, defined as < 8 g/dL[20]
3. Meeting the following symptom cluster exclusion for all eligible clusters[#]:
    a. Cognitive dysfunction: known stroke that resulted in cognitive impairment within 3 months of enrollment
    b. Autonomic dysfunction: atrial fibrillation or significant cardiac arrhythmia, more than moderate alcohol consumption[¥], pre-existing sustained severe hypertension (BP> 180/110 mmHg in the sitting position)
    c. Exercise intolerance:
        i. any of the following within 4 weeks of consent - an acute myocardial infarction or unstable angina, uncontrolled arrhythmias causing symptoms or hemodynamic compromise, acute myocarditis or pericarditis, uncontrolled acutely decompensated heart failure (acute pulmonary edema), acute pulmonary embolism, suspected dissecting aneurysm, severe hypoxemia at rest, any acute or chronic disorder that may affect exercise performance
        ii. if they are aggravated by exercise (e.g., infection, thyrotoxicosis, unable to cooperate)

[#]Participants who are eligible for > 1 cluster must meet all inclusion and no exclusion criteria for an individual symptom cluster. If not, they will be excluded from that individual symptom cluster.

[¥] Defined as greater than 2 drinks a day for men and 1 drink a day for women. A drink is equivalent to 12 ounces of beer (5% alcohol content), 8 ounces of malt liquor (7% alcohol content), 5 ounces of wine (12% alcohol content), 1.5 ounces or a "shot" of 80-proof (40% alcohol content) distilled spirits or liquor (e.g., gin, rum, vodka, whiskey). [21]

4. Known diagnosis of Lyme disease

5. Any non-marijuana illicit drug use within 30 days of informed consent
6. Current or recent use (within the last 14 days) of study intervention*
7. Known allergy/sensitivity or any hypersensitivity to components of the study intervention (s) or control*
8. Known contraindication(s) to study intervention(s),
9. Inability to discontinue symptomatic medications for the identified time periods (See Section 6.5.1)
10. Moderate or severe immunocompromised patients, such as those described in the NIH COVID-19 Treatment Guidelines (*https://www.covid19treatmentguidelines.nih.gov/special-populations/immunocompromised/*)
11. Enrolled into another study intervention appendix in this platform protocol[$]
    [$]*Participants may re-enroll in the trial for a different study intervention appendix if they have completed an appropriate washout period and efficacy has been determined for the appendix in which they were previously enrolled.*
12. Any condition that would make the participant, in the opinion of the investigator, unsuitable for the study

**If only one study intervention appendix is open at the time of enrollment. If multiple study intervention appendices are open, a participant may be excluded from any study intervention appendix based on contraindications listed in the study intervention appendix, current use of study intervention, or known allergy/sensitivity/hypersensitivity and still remain eligible for the remaining study intervention appendices.*

5.3 SPECIAL CONSIDERATIONS FOR PARTICIPANT ENROLLMENT

Participants will not be enrolled into a symptom cluster cohort for which they also meet symptom cluster specific exclusion criteria. Assessments for a symptom cluster will be performed among participants who meet the symptom cluster-specific eligibility criteria. Participants may meet eligibility criteria for more than one symptom cluster. In phase 2 appendices, they will undergo screening and post randomization assessments for all of the symptom clusters for which they qualify. In phase 3 appendices, assignment to a symptom cluster may be more limited. Refer to appendices for details.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

5.5 STUDY DEFINITION OF ENROLLMENT

For this study, enrollment is defined as signing consent and completing randomization.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

The RECOVER Clinical Trial Data Coordinating Center (CT-DCC) will use an integrated strategy of coordinating with community organizations, the public, and clinical trial sites to identify and retain study participants. To ensure a diverse population is enrolled, strategies from prior successful initiatives will be refined and utilized. The study team will develop a comprehensive communication strategy involving print and social media, as well as leveraging existing organizational structures where possible, to educate the public on concerns about PASC and opportunities for clinical trial participation. Interested members of the public will be provided with information to contact a local site for potential participation.

Participants can be recruited and identified through participating site outreach. Site investigators, or their designee, may contact eligible participants to introduce the study and discuss study participation.

Participants may be recruited from other ongoing COVID-19 trials if they opted-in to be contacted about future research opportunities. Patient advocates that represent a diverse PASC community will be engaged in the study at every step. Patient advocates will serve as consultants to inform study design, protocol development, and recruitment and retention strategies.

During active study, study sites will maintain close connections with study participants.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

See appendices.

6.1.2 DOSING AND ADMINISTRATION

See appendices.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Receipt and use of study drug will be handled, tracked by the sites, and stored in a safe, secure, and temperature-monitored location to which only the investigator and designated personnel have access. Use of study drug will be tracked by the sites. At minimum, the sites will check intervention adherence at the Drug Dosing Midpoint Clinic Visit to monitor compliance with study drug administration. Participants will also be asked to bring all unused drug with them to the clinic during their End of Dosing Visit.

See appendices for further details.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

See appendices.

6.2.3 PRODUCT STORAGE AND STABILITY

Upon receipt, study drug should be stored according to the instructions specified on the label. See appendices.

6.2.4 PREPARATION

See appendices.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

To achieve blinding and an equitable randomization probability, a two-step randomization process will be used.

At the first step, each participant will be assigned with equal probability to one of the appendices for which the participant is eligible after applying any appendix-level exclusion criteria. At the second step, if the appendix includes k dose levels of active intervention plus a control group, participants will be assigned with probability $1/(k+1)$ to one of the $(k+1)$ available treatment options.

For simplicity, the text below assumes that M_1 active appendices will have no sharing of control participants and that an additional M_2 active appendices each involve a single intervention dose level and will use a single pooled control group among them. Let k_i be the number of dose levels in the i-th appendix where no restriction is placed on $k_1, \ldots, k_{M_1}$ and $k_{M_1+1} = k_{M_1+2} = \cdots = k_{M_1+M_2} = 1$. Randomization will proceed as follows for a participant who is eligible for m_1 of the M_1 appendices with no sharing of controls and m_2 of the M_2 appendices with sharing of controls. At the first stage, the participant will be assigned with probability $1/(m_1+m_2)$ to one of the appendices for which the participant is eligible. If the randomly assigned intervention is unable to use a pooled control group, the second stage randomization will assign the participant with probability $1/(k_i+1)$ to one of the available intervention groups, where i denotes the randomly assigned appendix. If the randomly assigned intervention is one that will use a pooled control group, the second stage randomization will assign the participant to active intervention or control at an m_2: 1 ratio, where m_2 is defined above.

Sites will be informed to which study intervention appendix the participant is randomized, but not whether they are allocated to one of the active intervention dose levels or control within that appendix. The participants and investigators will be blinded throughout the study.

6.3.1 UNBLINDING

For each sub-study, the participant, treating clinicians, and study personnel will remain blinded to study intervention versus control assignment until after the database is locked and blinded analysis for that sub-study is completed. Blinding may continue beyond the final analysis if the control group data are

being used by another ongoing sub-study. Only the biostatistical team who is preparing closed interim reports will be unblinded. Specifically, study intervention/control will be dispensed with packaging and labelling that blinds treatment assignment. Unblinding will occur only if required for participant safety or treatment, at the request of the treating clinician. Refer to the Manual of Procedures (MOP) for further details.

6.4 STUDY INTERVENTION ADHERENCE

Participants will be notified of the importance of completing the full treatment course.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are relevant concomitant prescription medications, over-the-counter medications, and supplements. Baseline concomitant medications will include all concomitant therapies taken by the participant within 14 days of informed consent. Concomitant medications will be reviewed through the End of Study.

6.5.1 SYMPTOMATIC THERAPY

Drugs administered for symptomatic treatment and initiated prior to screening may be continued as long as there is no contraindication due to drug-drug interaction with the study intervention and there is no change in dose or administration during the study period. Administration of the following therapies for each symptom cluster will be carefully documented and closely monitored during the study period because of the potential for changes in these therapies to alter study results:

Cognitive dysfunction: prescribed or illicit stimulants, amantadine, N-methyl-D-aspartate receptor antagonists (e.g., memantine, dissociative drugs).

Autonomic dysfunction: ephedrine, midodrine, amphetamine/attention deficit hyperactivity disorder medications, tricyclic antidepressants, florinef, sympathomimetics, Adderall, methylphenidate, atomoxetine, or other serotonin and norepinephrine reuptake inhibitors.

Exercise intolerance: none

6.5.2 RESCUE MEDICINE

Participants who require a rescue medication to treat a non-study-related acute condition during the study period should proceed with treatment for the acute condition, as prescribed by their treating clinician. They may continue to receive study drug as long as the rescue medication is not listed as a contraindication in any of the active study appendices. If the rescue medication is contraindicated, the participant will discontinue the use of the study intervention, but will continue to be followed per the Schedule of Procedures (Table 2). These participants will be considered evaluable for the analysis.

7 PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 PARTICIPANT DISCONTINUATION FROM STUDY DRUG

Discontinuation from study intervention does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the appendix protocol. If a clinically significant finding is identified after enrollment, such as changes from baseline, the investigator or qualified designee will determine if any change in participant management is needed.

An investigator may discontinue a participant from the study intervention at their discretion, for any reason including, but not limited to, one of the following:

- Significant study intervention non-compliance
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study or intervention would not be in the best interest of the participant
- Confirmed new case of acute SARS-CoV-2

Participants who discontinue due to an AE will be followed until resolution of the AE. The reason for participant discontinuation from study intervention will be recorded on the CRF. Participants who are discontinued from the study intervention but not withdrawn from the study will continue to be followed for all study procedures. If a participant discontinues the study intervention, but does not withdraw consent, they will be followed for safety for at least 28 days after the final study intervention administration.

7.2 PARTICIPANT WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. The study team will attempt to determine a reason for withdrawal; however, participants are not obligated to provide a reason for withdrawal. If obtained, the reason for withdrawal will be recorded on the CRF. No further study procedures will be performed and no further data will be collected from the participant following study withdrawal. All of the data collected up until the time of withdrawal will be maintained in the study database and will be used as the participant's data are evaluable for analysis.

7.3 LOST TO FOLLOW-UP

Participants will be considered lost to follow-up if they fail to return for any scheduled visit *and* if they are unable to be contacted after multiple attempts and methods by the study site staff and/or a central search company.

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

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant or next of kin (where possible, telephone calls and,

if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, after exhausting all methods, the participant will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7.4 STUDY HALTING RULES

Study intervention dosing and enrollment will be temporarily suspended pending review of the Data and Safety Monitoring Board (DSMB) based on appendix-specific criteria. The DSMB recommendations will be considered by the NIH and the study Principal Investigator prior to making any decisions regarding study continuation or discontinuation.

8 STUDY ASSESSMENTS AND PROCEDURES FOR PHASE 2 STUDY DESIGNS

8.1 SCHEDULE OF PROCEDURES

Table 2. Schedule of study procedures

	Screening	Baseline	Drug Dosing Midpoint Clinic Visit	End of Dosing Clinic Visit	Follow-up Through day 179				End of Study Visit[4]
PROCEDURE	**Day -14 to 0**	**Day -2 to 0**	**Refer to appendix[1]**	**Day EOD + 3 days**	**Day 45 ± 5 days**	**Day 60 ± 5 days**	**Day 90[3] ± 5 days**	**Day 120 ± 5 days**	**Day 183 ± 7 Days**
Informed consent	X								
Demographics	X								
Medical History	X								
Concomitant Medications	X	X	X	X	X	X	X	X	X
PROMIS-29+2		X	Weekly[2]	X	Weekly[2]	Weekly[2]	X	Weekly[2]	X
PASC Symptom Questionnaire		X	Weekly[2]		Weekly[2]	Weekly[2]		Weekly[2]	
CTCSQ	X			X			X		X
Performance Measure Symptom Cluster Assessment(s) (Table 4)		X[8]		X[8]			X[8]		
ISWT (**Exercise Intolerance Cluster Only**)	X[8,9]								
PRO Symptom Cluster Assessment(s) (Table 4)	X		Weekly[2]	X	Weekly[2]	Weekly[2]	X	Weekly[2]	X
Appendix-Level Eligibility Criteria	X								
Pregnancy Test		X							
Blood Collection[5]		X	X	X	X[6]	X[6]	X	X[6]	
Nasal Swab		X	X[7]	X[7]	X[6,7]	X[6,7]	X[7]	X[6,7]	
Stool Collection		X					X		
Height and weight		X							
BP and Pulse (supine & standing)		X		X			X		
Randomization		X							
Study Drug Administration			X						
SAEs, SUSARs		X[10]	X[10]	X[10]	X	X	X	X	X
Healthcare Utilization			X	X			X		X
ESIs			X	X	X	X	X	X	X
Drug Adherence			X	X					
Underlined visits must be done in-person.									

[1]In-clinic evaluation at the drug dosing midpoint, defined per appendix
[2]Remote data collection approximately weekly during drug dosing and follow-up unless presenting to clinic for optional blood collection, as applicable. Participants will have 3 days to complete weekly assessments.
[3]Primary endpoint assessment
[4]End of Study Visit will occur by telephone

[5] Blood for clinical laboratory assessments, mechanistic evaluation, and biorepository storage for future study will be collected at baseline, drug dosing midpoint, end of dosing, and day 90 for all participants.
[6]Optional nasal swab and blood draws
[7]Nasal swab for RAT and PCR
[8]The DSQ-PEM must be completed remotely +1 to 2 days after any visit that includes Performance Measure Symptom Cluster Assessment(s) to assess PEM.
[9]For participants who are considered for the Exercise Intolerance Symptom Cluster, the ISWT must be completed at an in-person screening visit that occurs at least 3 days before the Baseline Visit.
[10]Non-serious AEs will be collected from the start of study drug administration through the EOD for select appendices.

8.2 SCREENING (DAY -14 TO 0)

Information about the study will be presented to potential participants and questions will be asked to determine potential eligibility. Study-specific screening procedures will begin only after informed consent is obtained.

Screening procedures may be done over one or two calendar days. However, in many cases (e.g., autonomic dysfunction and cognitive dysfunction symptom clusters) all the screening assessments can be done in < 24 hours. If that is the case, baseline assessments, specimen collection, and the initial study product administration can occur on the same calendar day.

After the informed consent, the following assessments will be performed at screening to determine eligibility:

- Demographics
- Medical History; including SARS-CoV-2 vaccination status and dates, SARS-CoV-2 test result date (if available), signs and symptoms, and treatment (including hospitalization, Intensive Care Unit (ICU) status, supplemental O_2 status) and PASC history (symptoms and duration)
- Collection of concomitant medications taken within 14 days of informed consent
- CTCSQ
- ISWT; for participants who are considered for the Exercise Intolerance Symptom Cluster, the ISWT must be completed at an in-person screening visit that occurs at least 3 days before the Baseline Visit. The DSQ-PEM must be completed remotely +1 to 2 days after the visit where ISWT is performed to assess PEM.
- PRO Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments
- Review appendix-level eligibility criteria, refer to appendices

8.3 BASELINE (DAY -2 TO 0)

Baseline assessments will preferably occur on the same day as the first intervention administration and may occur on the same day as Screening. In the event that drug dosing does not begin on the same day as the Baseline visit, intervention administration must begin within 48 hours of baseline assessments. The following will occur at the Baseline visit:

- Concomitant Medication review

- PROMIS-29+2
- PASC Symptom Questionnaire
- Performance Measure Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments. The DSQ-PEM must be completed remotely +1 to 2 days after the visit to assess PEM.
- Pregnancy test, for individuals capable of becoming pregnant, blood or urine
- Blood Collection for the following evaluations:
    - Local: Comprehensive metabolic panel (if not done within the past 3 months), high-sensitivity c-reactive protein, D-dimer, coagulation panel (prothrombin time, activated partial thromboplastin time, thrombin time), complete blood count with differential (if not done within the past 3 months)
    - Send out: Mechanistic evaluation and biorepository storage for future investigation
    - Safety labs, per appendix (local)
- Nasal Swab for SARS-CoV-2 rapid antigen test (RAT), completed locally, and polymerase chain reaction (PCR), completed at central lab
- Stool Collection for mechanistic evaluation, processed at central lab
- Height and Weight
- Blood Pressure (BP) and Pulse, supine and standing
- Randomization
- Safety assessment including SAEs and suspected unexpected serious adverse reactions (SUSARs), non-serious AE from the start of study drug administration for select appendices

8.4 DRUG DOSING MIDPOINT CLINIC VISIT

Study intervention dosing may begin on the same day as the Baseline Visit, but must occur within 48 hours of baseline assessments. Refer to appendices for intervention-specific dosing duration.

At approximately the midpoint of drug dosing, a Drug Dosing Midpoint Clinic Visit will occur. Refer to the appendices for the timing of the midpoint visit. At this visit, the following will occur:

- Concomitant Medication review
- Study drug administration, per study drug appendix
- PROMIS-29+2, approximately weekly remotely outside of the in-person visit
- PASC Symptom Questionnaire, approximately weekly remotely outside of the in-person visit
- PRO Symptom Cluster Assessment(s), approximately weekly remotely outside of the in-person visit. Refer to Section 8.10.4 for Symptom Cluster Assessments
- Blood Collection, for the following evaluations:
    - High-sensitivity c-reactive protein, D-dimer, coagulation panel (prothrombin time, activated partial thromboplastin time, thrombin time), complete blood count with differential (local)
    - Mechanistic evaluation
    - Safety labs, per appendix

- Nasal Swab for SARS-CoV-2 RAT, completed locally, and PCR, completed at central lab
- Safety assessment including SAEs, SUSARs, and ESIs; non-serious AE for select appendices
- Healthcare Utilization
- Drug Adherence

8.5 END OF DOSING CLINIC VISIT (DAY END OF DOSING + 3 DAYS)

The End of Dosing Clinic Visit will occur after the final intervention dose has been administered. The following will occur at the End of Dosing Clinic Visit:

- Concomitant Medication review
- PROMIS-29+2
- PASC Symptom Questionnaire
- CTCSQ
- Performance Measure Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments. The DSQ-PEM must be completed remotely +1 to 2 days after the visit to assess PEM.
- PRO Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments
- Blood Collection, for the following evaluations:
    - High-sensitivity c-reactive protein, D-dimer, coagulation panel (prothrombin time, activated partial thromboplastin time, thrombin time), complete blood count with differential (local)
    - Mechanistic evaluation
    - Biorepository storage for future study
    - Safety labs, per appendix
- Nasal Swab for SARS-CoV-2 RAT, completed locally, and PCR, completed at central lab
- Blood Pressure (BP) and Pulse, supine and standing
- Safety assessment including SAEs, SUSARs, and ESIs; non-serious AEs for select appendices
- Healthcare Utilization
- Drug Adherence evaluation based on any doses returned at this visit

8.6 FOLLOW-UP [END OF DOSING (+3 DAYS) TO DAY 179]

The Follow-Up Period will begin after the End of Dosing Clinic Visit. A follow-up clinic visit will occur for all participants on Day 90 (± 5 days). All assessments required for the Day 90 visit will occur at the Day 90 (± 5 days) in-person visit; any assessments completed remotely during the week of the Day 90 visit cannot replace the in-person Day 90 assessments. All participants will receive remote follow-up approximately weekly with optional in-person visits for blood collection on Days 45 (± 5), 60 (± 5), and 120 (± 5) days. All assessments that are completed remotely on a weekly basis will be open to the participant to complete for 3 days. Any assessments that are not completed within that window will expire and will not be collected. The following assessments will occur as part of follow-up:

- Concomitant Medication review

- PROMIS-29+2
- PASC Symptom Questionnaire + global health question #13 from the CTCSQ
- CTCSQ (**Day 90 only**)
- PRO Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments
- Performance Measure Symptom Cluster Assessment(s). Refer to Section 8.10.4 for Symptom Cluster Assessments (**Day 90 only**). The DSQ-PEM must be completed remotely +1 to 2 days after the visit to assess PEM.
- Blood Collection, for the following evaluations:
    - High-sensitivity c-reactive protein, D-dimer, coagulation panel (prothrombin time, activated partial thromboplastin time, thrombin time), complete blood count with differential (local, **Day 90 only**)
    - Mechanistic evaluation (**required on Day 90, optional on Day 45, 60, and 120**)
    - Biorepository storage for future study (**required on Day 90, optional on Day 45, 60, and 120**)
    - Safety labs, per appendix (local, **Day 90 only**)
- Nasal swab for SARS-CoV-2 RAT, completed locally, and PCR, completed at central lab (**required on Day 90, optional on Day 45, 60, and 120**)
- Stool Collection (**Day 90 only**), processed at central lab
- Blood Pressure (BP) and Pulse, supine and standing (**Day 90 only**)
- Healthcare Utilization (**Day 90 only**)
- Safety assessment including SAEs, SUSARs, and ESIs

8.7 END OF STUDY VISIT (DAY 183 ± 7 DAYS)

The End of Study Visit will be a remote visit. The following will occur at the End of Study Visit:

- Concomitant Medication review
- PROMIS-29+2
- CTCSQ
- PRO Symptom Cluster Assessment(s) based on participant cluster assignment. Refer to Section 8.10.4 for Symptom Cluster Assessments
- Healthcare Utilization
- Safety assessment including SAEs, SUSARs, and ESIs

8.8 OPTIONAL BLOOD COLLECTION/NASAL SWAB VISITS

Participants will be asked to attend optional Follow-up Visits that include blood collection and nasal swabs at Days 45 (± 5 days), 60 (± 5 days), and 120 (± 5 days). Participants opting to attend these optional visits will be compensated for their time and effort.

8.9 CLINICAL LABORATORY ASSESSMENTS

A comprehensive metabolic panel, high-sensitivity c-reactive protein, D-dimer, coagulation panel (prothrombin time, activated partial thromboplastin time, thrombin time), complete blood count with differential will be completed as local laboratory assessments at baseline, the drug dosing midpoint clinic visit, the end of dosing clinic visit, and at the day 90 follow-up visit.

For the baseline clinical laboratory assessments, if a participant has these laboratory assessments available within 3 months of study enrollment, they do not need to be repeated as part of the study. Additional clinical laboratory assessments may be required per appendix, refer to appendices.

The SARS-CoV-2 RAT will also be completed locally according to the timepoints indicated in Table 2.

8.10 STUDY ASSESSMENTS

8.10.1 BIOSPECIMEN COLLECTION

Because a primary focus of this protocol will be to investigate mechanisms underlying PASC and improvement with antivirals and other therapies, select biomarkers will be evaluated over time among those who receive intervention or control. These biomarkers are targeted towards the leading hypotheses for PASC pathogenesis, including viral persistence/reactivation, immune dysregulation, and dysregulation of coagulation and will be obtained from blood (~80 ml), stool, and nasal swabs.

Biomarkers will be collected at various time points that will enable the understanding of potential improvement with therapy and rebound, and will be collected at similar time points as PROs and other assessments in order to understand the relationship between the biomarkers and clinical findings.

At minimum, the following biomarkers will be assessed:

- Antigenemia (SARS-CoV-2 antigens), including the S1 subunit of spike, full length spike, and nucleocapsid in plasma

While some important biomarkers will be known at the time of participant enrollment and assays will be available, others may not. In addition to biomarkers, peripheral blood mononuclear cells will be collected at *select sites*. Samples collected throughout the course of this study will be stored at the RECOVER Biorepository, which is designed to collect and store biospecimens for future research related to the various studies of the RECOVER Program. Samples from biorepositories have proven to be enormously important in the last 20 years, as information on the components of blood has expanded rapidly. Important insights have been gained from biorepository samples from clinical trials and the stored samples from the RECOVER Program will prove equally productive and important. This biorepository will be conducted under the coordination of the Duke Clinical Research Institute (DCRI) which serves as the CT - DCC for all RECOVER clinical trials.

Samples will be collected for storage at -80°C and will be stored at the Biorepository in a lab at the Mayo Clinic in Rochester, MN for up to 7 years.

8.10.2 PROMIS-29+2 (PROPR)

Patient-Reported Outcomes Measurement Information System (PROMIS-29) global health scale: The PROMIS was developed out of the "Roadmap for Medical Research" created by the NIH in 2002 as valid, generalizable items to standardize clinical research across NIH-funded research dealing with PROs. Multiple PROMIS scales have been validated across many clinical populations.[22] The PROMIS-29 consists of 29 items that assess general domains of health and functioning, including overall physical health, mental health, social health, pain, fatigue, and overall perceived quality of life. The PROMIS global health scales has been correlated against the EuroQol EQ-5D. [23] Additionally, PROMIS scales have been used with PASC patients.[24]

The PROMIS-29+2 is used to calculate a preference score (PROPr) by the addition of two Cognitive Function Ability items. Preference-based scores provide an overall summary of HRQOL on a common metric. Preference-based scores summarize multiple domains on a metric ranging from 0 (as bad as dead) to 1 (perfect or ideal health). Scores can be used in comparisons across groups and for cost-utility analyses. [25]

8.10.3 CLUSTER TARGETED COVID-19 SYMPTOM QUESTIONS (CTCSQ)

The CTCSQ is a set of thirteen questions with the first twelve items rated none, mild, moderate, or severe. The final item is a global symptom question. Participants must report having at least two moderate symptom(s) from the same Symptom Cluster or one severe symptom in order to meet eligibility criteria. If a participant reports a moderate or severe item #1-3, they will complete the exercise intolerance and autonomic dysfunction cluster assessments at Baseline. If a participant reports a moderate or severe item #4-8, they will complete the autonomic dysfunction cluster assessments at Baseline. If a participant reports a moderate or severe item #9-12, they will complete the cognitive dysfunction cluster assessments at Baseline. Item #13 is not associated with specific symptom clusters and will not have any influence on symptom cluster assignment. After cluster assignment, the CTCSQ will not result in any alterations to cluster assignment, but will be used as a symptom tracking tool.

Table 3. CTCSQ items #1-13 and associated symptom cluster(s)

Please rate the level of severity you experienced of the following symptoms in the last 7 days:	None	Mild	Moderate	Severe	Associated Symptom Cluster
1. Fatigue (being tired)					Exercise Intolerance/ Autonomic Dysfunction
2. Post-exertional malaise (symptoms worse after even minor physical or mental effort)					
3. Weakness in your arms and/or legs					
4. Pain in your limbs					Autonomic Dysfunction
5. Faintness (light-headedness)					
6. Gastrointestinal upset					
7. Color changes of the skin					

8. Dry mouth					
9. Problems with thinking or concentrating (brain fog)					Cognitive Dysfunction
10. Confusion					
11. Forgetfulness					
12. Difficulty focusing					
GLOBAL QUESTION	Improved a lot	Improved somewhat	Stayed about the same	Worsened somewhat	Worsened a lot
13. Compared to the start of the study, the overall severity of your Long COVID symptoms have:					

8.10.4 SYMPTOM CLUSTER ASSESSMENT

As part of screening, potential participants will answer the CTCSQ. Eligible participants will complete Symptom Cluster assessments based on their CTCSQ responses (see Table 3) and will subsequently be assigned to one or more of the three Symptom Clusters based on the baseline assessments. Participants must meet criteria for the PRO within a specific symptom cluster in order to be included in the cluster, see *italicized* text within each cluster for inclusion reference points. After study enrollment and initial cluster assignment, further assessments will be performed among participants who meet the symptom cluster criteria. Participants whose answers qualify them for additional screening for multiple Symptom Clusters will undergo assessments for those Symptom Clusters. Refer to Figure 3.

Table 4. Symptom cluster assessments schedule

	Screening	**Baseline**	**Drug Dosing**	**End of Dosing Clinic Visit**	**Follow-up Through day 179**				**End of Study Visit**	
PROCEDURE	**Day -7 to 0**	**Day -2 to 0**	**Day 0 to EOD**	**Day EOD + 3 days**	**Day 45 ± 5 days**	**Day 60 ± 5 days**	**Day 90 ± 5 days**	**Day 120 ± 5 days**	**Day 183 ± 7 Days**	
DSQ-PEM (+1 to 2 days following visit where Performance Measures are completed)		X		X			X			All Clusters
DSQ-PEM	X	X	Weekly[1]		Weekly[2]				X	Exercise Intolerance
ISWT	X									
ESWT		X		X			X			
Cardiopulmonary test (**select sites**)		X		X			X			
PROMIS-Cognitive 8a	X		Weekly[1]		Weekly[2]				X	Cognitive Dysfunc
ECOG 2	X		Weekly[1]		Weekly[2]				X	
Neurocognitive battery		X		X			X			
COMPASS 31	X		Weekly[1]		Weekly[2]				X	Autonomic
OHQ	X		Weekly[1]		Weekly[2]				X	

Active Stand Test		X		X			X			
Full Autonomic Testing (**select sites**)		X		X			X			

[1]Must also be completed at the Drug Dosing Midpoint Clinic Visit
[2]Must also be completed at the Day 90 ± 5 days in-person visit

- Cognitive dysfunction:
    - PROMIS – cognitive 8a[26] (PRO assessment). Part of the PROMIS test battery, the cognitive function is a quantitative measure of current cognitive function. *A PROMIS – cognitive 8a function T-score < 40 will include a participant in this cluster and trigger other baseline assessments listed below*.
    - ECOG 2 (PRO assessment). The Everyday Cognition 2 is a self-report, 41-item questionnaire used to measure the participant's perceived capacity to perform activities related to cognitive function, which could impact major activities of daily living and independence. It has been used for patients with mild cognitive impairment, Alzheimer's Disease, and dementia. It takes approximately 15 minutes to complete.
    - Neurocognitive battery (performance measure) to assess various elements related to cognition. The neurocognitive battery consists of a cognitive assessment sequence of the following: WHO/UCLA Auditory Verbal Learning Test (WHO/UCLA AVLT) and Symbol Digit Modalities Test. The entire battery takes approximately 30 minutes to complete.
- Autonomic dysfunction:
    - COMPASS 31[27] (PRO assessment). COMPASS 31 is a measure of autonomic symptoms across multiple domains commonly seen in patients with PASC. *A COMPASS 31 score ≥ 25 in combination with OHQ requirements will include a participant in this cluster.*
    - Orthostatic Hypotension Questionnaire (OHQ) [PRO assessment]. The OHQ is a measure of orthostatic intolerance, which has been the primary presentation of patients with PASC-related autonomic dysfunction. This measure includes the Orthostatic Intolerance Daily Activity Scale (OIDAS) and the Orthostatic Intolerance Symptom Assessment (OISA), and prior data in patients with PASC suggests that this measure well-discriminates those with symptoms compared to healthy controls. For this study and to reduce participant burden, the participants will be asked to complete the OIDAS and to rate only their most severe symptom on the OISA each time they complete the OHQ. At each visit where the OHQ is conducted, participants will complete the OHQ at the start of the visit and then repeat it at approximately 30 minutes after completing all other visit activities. *A value ≥ 4 on question 1 of the OISA of the OHQ in combination with COMPASS 31 requirements will include a participant in this cluster.*
    - Active stand test (performance measure). The active stand test is a well-characterized measure of short-term neurologic and cardiovascular function that requires limited resources and aids in diagnosis of patient symptoms of orthostatic intolerance.
    - Full autonomic testing (exploratory performance measure) consists of two catecholamine collections, respiratory sinus arrhythmia, hyperventilation, Valsalva

maneuver, and a head up tilt test and will be completed at select sites. The entire battery takes approximately 90 minutes to complete.

- Exercise intolerance:
    - The Modified DePaul Symptom Questionnaire Post Exertional Malaise (DSQ-PEM) PRO assessment.[28] Some patients have experienced debilitating PEM that may occur following exertion or on the day after exertion. To assess PEM, the DSQ-PEM short form will be adapted. This scale assesses symptom frequency and severity over a 6-month look back period; however, for the purposes of this study, it will be modified to assess over a 1-week look back period. It was previously validated in patients with myalgic encephalomyelitis/chronic fatigue syndrome. Frequency is rated on a 5-point Likert scale: 0 = none of the time, 1 = a little of the time, 2 = about half the time, 3 = most of the time, and 4 = all of the time. Severity is also rated on a 5-point Likert scale: 0 = symptom not present, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe. Post-Exertional Malaise (PEM) will be deemed to be present for participants reporting at least one moderate (rated severity ≥ 2) PEM symptom for a frequency rated ≥ 2. *At least one moderate symptom (severity ≥ 2, frequency ≥ 2) on the DSQ-PEM in questions 1-5 will include a participant in this cluster and trigger other baseline assessments listed below.*
    - Endurance shuttle walk test (performance measure). The ESWT consists of timed walking on a 10 meter course.[29] The result is expressed as total walking time after an initial 2-minute warm-up. In order to determine walking speed, the baseline ESWT will be performed in combination with the ISWT.[30] Walking speed for the ISWT will increase every minute until the participant is too breathless or fatigued to continue walking at the required speed. The ESWT will then be performed as 85% of the maximum walking speed achieved on the ISWT.[29]
    - Cardiopulmonary exercise testing (exploratory performance measure) will be completed at select sites. This is an exercise test that measures exercise abilities. The test involves a 2 to 3-minute warm-up, followed by 8 to 12 minutes of progressively harder work on a treadmill or stationary bike. The goal is to exercise until the participant is no longer able to continue. The final outcome for this measure will be the maximum rate of oxygen consumption (VO2 max).
- All Clusters
    - The DSQ-PEM will be performed remotely approximately 1-day (+1 day) after assessment of Performance Measures. For the purposes of assessing PEM related to the specific activity, it will be modified to assess PEM related specifically to the activity that occurred the previous day.

Each assessment will occur according to Table 2 and Table 4. Procedures may be completed during the study as part of regular standard of clinical care. Training to standardize these assessments will be provided to participating sites by the RECOVER CT-DCC and protocol study teams.

8.10.5 PASC SYMPTOM QUESTIONNAIRE

Participants will be asked to complete a questionnaire that asks about the presence of PASC symptoms. This questionnaire includes additional symptoms that are not directly related to the Symptom Clusters in this study.

9 SAFETY ASSESSMENTS AND REPORTING

9.1 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

9.1.1 DEFINITION OF ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

An AE is any untoward medical occurrence in humans, whether or not considered drug-related, which occurs during the conduct of a clinical trial. An AE can therefore be any change in clinical status, routine labs, x-rays, physical examinations, etc., that is considered clinically significant by the study investigator.

An SAE or serious suspected adverse reaction (SAR) or serious adverse reaction, as determined by the investigator or the sponsor, is an AE that results in any of the following serious outcomes:

- Death
- Life-threatening AE ("life-threatening" means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred and required immediate intervention)
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Inpatient hospitalization or prolongation of existing hospitalization
- Congenital abnormality or birth defect
- Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline does not meet the definition of an SAE. Hospitalization is defined as a stay in the hospital exceeding 24 hours.

An unexpected AE is defined as any AE, the specificity or severity of which is not consistent with the study drugs' package insert or investigators brochure.

An unanticipated problem involving risk to participants or non-participants, including an environmental exposure and exposure to a breastfeeding infant, will be reported to the sponsor and the site IRB and central IRB, as appropriate. The event occurring in the non-participant will not be entered into the electronic data capture (EDC) system. Refer to the MOP and/or Safety Management Plan (SMP) for details.

Medication errors with the study drug(s) resulting in an SAE are reportable. The medication error will be captured as a protocol deviation and the SAE captured on the SAE electronic case report form (eCRF).

9.1.2 COLLECTION PERIOD FOR AE AND SAE INFORMATION

Safety event collection will occur at the pre-specified study visits but all participants will be instructed to self-report concerns by calling the site.

Serious adverse events (SAEs) or ESIs (per appendix) will be extracted by site personnel from the participant's medical record if the participant seeks medical care or if hospitalization occurs, each of which notifies the site to conduct follow-up.

Medical occurrences that begin before the first invasive study procedure (blood collection or nasal swab), but after obtaining informed consent, will not be considered an AE. The medical occurrence or condition will be captured on the Medical History eCRF.

Non-serious AEs that result in study drug discontinuation will be identified as the reason for study drug discontinuation within the study database, reported as an AE and collected from the start of study drug administration through the end of study drug dosing. Each study drug appendix will identify whether additional non-serious AEs require collection and reporting such as spontaneously reported non-serious AEs by the participant at clinic visits that occur during the dosing period, but do not result in study drug discontinuation. There is no expectation nor plan by the site nor the DCC to reconcile AEs reported by the participant with the patient reported outcome survey.

Serious adverse events (SAEs) will be collected from the first invasive study procedure (blood collection or nasal swab) through the End of Study Clinic Visit (Day 183 ± 7 days).

Adverse events (AEs) that qualify as an ESI, even if a non-serious AE, will be collected from the start of study drug administration through the End of Study Clinic Visit (Day 183 ± 7 days).

9.1.2.1 SEVERITY OF EVENT

For reportable events, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious" for regulatory reporting

9.1.2.2 RELATIONSHIP TO STUDY INTERVENTION

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

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study

intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE

- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established

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

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

All reportable events will be captured on the appropriate CRF. Information to be collected includes event description, date/time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), action taken with study drug (e.g. study drug discontinuation), and date/time of resolution/stabilization of the event. All events occurring within the pre-specified reportable time windows must be documented appropriately regardless of relationship.

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

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

The investigator will follow all SAEs until resolution, stabilization, or the event is otherwise explained. The DCRI Safety Surveillance Team will follow all SAEs until resolution, stabilization, or until otherwise explained.

9.1.4 REPORTING AND MONITORING OF SAES

All of the study drugs used in this platform protocol will be under an IND and subject to IND regulations in 21 CFR 312 since their investigational use for treatment of PASC is not an approved indication. The IND sponsor, DSMB, or Study Medical Monitor will review aggregate safety data. The IND sponsor will be responsible for determining if the safety reporting criteria are met per 21 CFR 312.32(c)(1)(i)(C) and 21 CFR 312.32(c)(1)(iv) and will notify the CT-DCC to prepare an aggregate report for submission to the FDA. An aggregate safety report will be submitted to FDA as soon as possible, but in no case later than 15 calendar days after the IND sponsor determination. If the IND sponsor determines that an unexpected fatal or life-threatening SAR occurs markedly more frequently in a study drug arm than in the control arm, an aggregate safety report will be submitted to the FDA as soon as possible, but in no case later than 7 calendar days after the IND sponsor determination. Information on individual SAEs will be available upon request from the FDA following the submission of any aggregate reports.

Individual SAEs must be entered into the data system within 24 hours of site awareness. The DCRI Safety Surveillance team will notify pharmaceutical partners of SAEs within 1 to 2 business days of their receipt that occur involving the specific appendix of the supplied study drug/control, as required. Serious Adverse Events that are related and confirmed unlisted by the DCRI Safety Medical Monitor and IND

sponsor will be reported to the FDA as SUSARs; as 7-day reports for unexpected fatal or life-threatening adverse reactions and 15-day reports for serious and unexpected adverse reactions. The SUSARs will be shared with the pharmaceutical partner of the supplied study drug according to the same timelines. If the IND sponsor, DSMB, or FDA note a clinically important increase in the rate of a SUSAR, the IND sponsor or designee will notify investigators no later than 15 calendar days after determining that the information qualifies for reporting. The investigators will notify their local IRB according to local guidelines if applicable. The CT-DCC will notify the central IRB. Refer to the Safety Management Plan for details regarding specific reporting timelines.

Investigators are not obligated to actively seek information on AEs or SAEs after the participant has concluded study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has completed the study (but before the study itself has ended), and they consider the event to be reasonably related to the study intervention, the investigator must promptly report the SAE to the sponsor via EDC entry.

9.1.5 EVENTS OF SPECIAL INTEREST

Each study drug may have a unique list of ESIs. Refer to the relevant Appendix.

9.1.6 REPORTING OF PREGNANCY

Pregnancies that occur following the first study drug administration while on-study will be collected. If included in the study drug appendix that pregnant participants are excluded, the participant will be advised to discontinue study drug(s). Pregnancies will be followed until pregnancy outcome unless the participant refuses to provide consent. Pregnant participants will be asked to complete a pregnancy-specific consent form if the final outcome of the pregnancy is not reached while the participant is on-study. Any pregnancy-associated ESI or SAE should be reported if information can be collected and entered into the CRF. The DCRI Safety Surveillance team will notify study drug supplying partners of a pregnancy within 1 to 2 business days after learning about the pregnancy, as required.

10 STATISTICAL CONSIDERATIONS FOR PHASE 2 DESIGN

10.1 STATISTICAL HYPOTHESES

Primary Endpoint:

- Improvement in symptom cluster-specific patient-reported outcome measures, including the DSQ-PEM, PROMIS-cognitive 8a, or OHQ at Day 90 compared to baseline in intervention(s) vs. control

For each pairwise comparison between an intervention and control group, the primary null hypothesis to be tested is that the probability of meeting a specified degree of improvement in symptom cluster-specific primary outcome measures (including the DSQ-PEM, PROMIS-cognitive 8a, or OHQ) at Day 90 compared to baseline is the same for intervention and control. The alternative hypothesis is that the probability of meeting a specified degree of improvement in symptom cluster-specific primary outcome measures at Day 90 compared to baseline differs for intervention and control. If π_D denotes the

probability of improvement if randomized to intervention and π_C denotes probability of improvement if randomized to control, then the primary null and alternative hypotheses may be expressed as follows:

$$H_0: \pi_D - \pi_C = 0 \quad \text{versus} \quad H_1: \pi_D - \pi_C \neq 0.$$

Secondary:

- Improvement in performance-based outcomes, including the ESWT, active stand test, and/or neurocognitive battery in intervention(s) vs. control
- intervention(s) are safe in the PASC population

The null hypothesis to be tested is that there is no difference between intervention and control group.

Exploratory:

- Improvement in viral, immune, and other mechanistic biomarkers over the course of the study in intervention(s) vs. control

The null hypothesis to be tested is that there is no difference between intervention and control group.

10.2 PRIMARY ESTIMAND

A separate pairwise comparison between intervention and control will be performed for each combination of study appendix, dose level, and symptom cluster. For each of these, the primary estimand will be the intention-to-treat treat between-group difference in marginal probabilities of improvement, defined as

$$\Delta_{\text{ITT}} = P(Y = 1|A = 1) - P(Y = 1|A = 0),$$

where Y denotes the primary outcome (1 = improvement, 0 = no improvement) and A denotes randomization assignment (1 = active treatment, 0 = control). This quantity will be estimated without regard to adherence and will implicitly average over observed patterns of concomitant therapies. In the unlikely event that follow-up data collection is prevented by death or hospitalization, the participant will be classified as not improving. The trial's SAP will provide further clarification of Δ_{ITT} and may specify additional secondary and supplementary estimands, for example, the hypothetical ideal between-group difference that would be observed in a setting of high treatment adherence.

10.3 SAMPLE SIZE DETERMINATION

This study uses an adaptive platform trial design that will allow study interventions to be added or dropped from consideration based on accruing evidence of futility or efficacy. In such a design, the required sample size depends on both the number of interventions tested and the ability to pool their control arms for analysis. Initial sample size estimates are based on a single appendix with a 1:1:1 allocation to each of two dose levels of an active intervention and control. If additional study interventions are added later that can contribute to pooled control, the sample size will be adjusted accordingly.

The sample size is based on a design that will evaluate treatment effects separately within three co-primary symptom cluster populations (cognitive dysfunction, autonomic dysfunction, and exercise intolerance) with a Holm-type adjustment to control the overall type-I error across them (see Section 10.5.1 for details). The Holm procedure will initially allocate alpha equally across symptom clusters (0.0166, 0.0166, 0.0166), with a total type-I error probability of 0.05. If the null hypothesis is initially

rejected for some but not all symptom clusters, the Holm procedure will allow the non-rejected hypotheses to be re-tested at an updated, less stringent significance level.

Accounting for the Holm procedure's stepwise alpha selection in power calculations is complicated because the final significance threshold is partly random and depends on the unknown configuration of treatment effects across all 3 symptom clusters considered jointly. As a simple conservative approximation, power was calculated for each symptom cluster individually assuming a specified treatment effect for that single symptom cluster and assuming 0.0166 for the Holm procedure's final significance threshold.

Analysis within each symptom cluster population will control the type-I error across multiple dose levels by analyzing them in a pre-specified hierarchical testing procedure in order from highest to lowest dose. For simplicity, the total sample size was obtained by calculating sample size for a simple two-group comparison and multiplying by 3/2.

Sample size was chosen to provide 80% power to detect a difference of 25 percentage points in the proportion of participants who experience the binary primary outcome for each pairwise comparison between a treatment group and control within each symptom cluster, assuming a two-sided test with alpha = 0.0166. Specifically, a sample size of 82 per group yields 80% power assuming the true outcome probabilities are 37.5% in the control group and 62.5% in the active treatment group. This sample size was inflated by 1/0.82 = 1.22 to account for potential 18% dropout/loss to follow-up.

The final required sample size per symptom cluster was calculated as 82 x 3 x 1.22 = 300. The total number of participants that will need to be randomized across 3 symptom cluster populations will depend on the proportion of participants who meet criteria for >1 symptom cluster population. Assuming no overlap, the maximum sample size will be 900 (300 × 3).

Table 5 illustrates the magnitude of treatment effects that will be detectable with 80% or 90% power under the study's planned sample size if the primary outcome occurs with probabilities ranging from 0.05 to 0.35 in the placebo group.

Table 5. Minimum treatment effects for 80% or 90% power

Probability of Improvement with Placebo	Minimum Treatment Effect Difference for 80% Power*	Minimum Treatment Effect Difference for 90% Power*
0.05	0.17 (0.22 vs 0.05)	0.20 (0.25 vs 0.05)
0.10	0.20 (0.30 vs 0.10)	0.23 (0.33 vs 0.10)
0.15	0.22 (0.37 vs 0.15)	0.25 (0.40 vs 0.15)
0.20	0.23 (0.43 vs 0.20)	0.27 (0.47 vs 0.20)
0.25	0.24 (0.49 vs 0.25)	0.28 (0.53 vs 0.25)
0.30	0.25 (0.55 vs 0.30)	0.28 (0.58 vs 0.30)
0.35	0.25 (0.60 vs 0.35)	0.28 (0.63 vs 0.35)

* Power for pairwise comparison between single dose level of active intervention versus control assuming 100 participants per treatment group (82 participants per treatment group with non-missing outcome data) and two-sided alpha = 0.0166.

10.4 POPULATIONS FOR ANALYSES

The primary analysis of all study data will be based on a modified intent-to-treat population (mITT), consisting of all randomized participants who receive at least part of one dose of the study intervention or control. The mITT population will be used when analyzing efficacy as well as safety outcomes. Participants will be analyzed according to their randomly assigned intervention group. A separate mITT population will be defined for each pairwise comparison between treatment groups within each co-primary symptom cluster population.

10.5 STATISTICAL ANALYSES

This section describes analysis methods for primary, secondary, and exploratory efficacy outcomes. Unless otherwise specified, analysis will be based on the mITT population. Full details will be provided in the SAP.

Because each sub-study is designed to address a separate set of questions pertaining to distinct agents, no multiplicity adjustments will be made across sub-study appendices. Plans for controlling type-I error within each sub-study are described below.

For each appendix, a separate analysis of the primary outcome will be performed in parallel for each of three primary symptom cluster populations. Participants who meet criteria for > 1 symptom cluster will be included in the data collection and analysis for all symptom clusters for which criteria are met. A Holm-type multiplicity adjustment will be used to control the type-I error probability at 0.05 for analysis of primary outcomes across the 3 symptom clusters. If there are multiple dose levels of an intervention, analysis of primary outcomes will be performed sequentially in order from highest to lowest dose. At each dose level, if the comparison between active intervention versus control is significant for all 3 symptom clusters, then testing of primary outcomes will proceed to the next lower dose level. Otherwise, testing will stop and primary outcome comparisons at lower dose levels will be regarded as exploratory. Comparisons between active intervention dose levels will be performed last and will be regarded as exploratory.

Figure 4 illustrates the protocol's multiple testing strategy for an appendix with two active dose levels. Hypotheses are grouped into a family $\mathcal{F}_1 = \{H_{11}, H_{12}, H_{13}\}$ for the higher dose level and a family $\mathcal{F}_2 = \{H_{21}, H_{22}, H_{23}\}$ for the lower dose level. Here, H_{dc} refers to the primary outcome analysis for symptom cluster c at dose level d. Testing begins with $\mathcal{F}_1$ and initially assigns local significance level α/3 to each member of $\mathcal{F}_1$. After rejecting a hypothesis within $\mathcal{F}_1$, its local alpha is reallocated equally within $\mathcal{F}_1$. In the event that all hypotheses within $\mathcal{F}_1$ are rejected, testing proceeds to $\mathcal{F}_2$, with alpha reallocated equally among its component hypotheses. In general, an appendix with $D \geq 1$ dose levels will test families $\mathcal{F}_1, \mathcal{F}_2, \ldots, \mathcal{F}_D$ in order from highest to lowest dose and will proceed to testing $\mathcal{F}_{d+1}$ if and only if all hypotheses within $\mathcal{F}_d$ have been rejected.

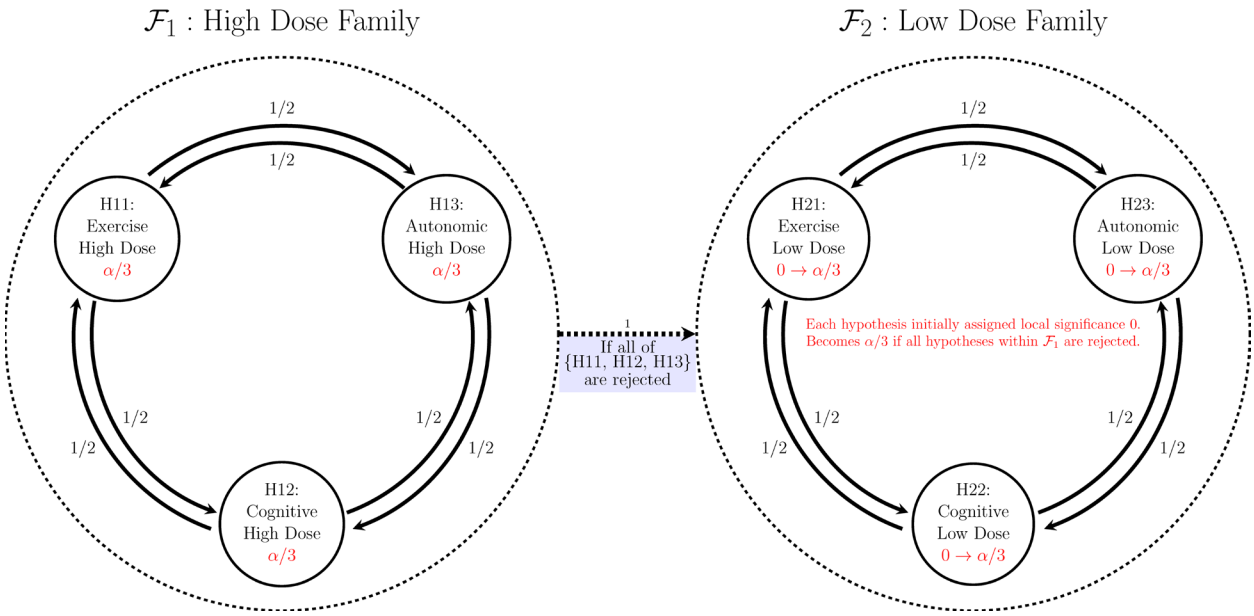

Figure 4. Multiple testing flowchart

The multiplicity procedure described above provides strong family-wise type-I error control across 3 symptom clusters and D dose levels for the primary outcomes. For secondary outcomes, the multiple testing strategy is less conservative. For each symptom cluster and dose level, if the hypothesis pertaining to the primary outcome is rejected, then the symptom cluster's major secondary outcome will be tested at the same significance level as the primary outcome. This does not strictly control type-I error across all primary and secondary outcomes, but does offer some protection by using the primary outcome as a gatekeeper for secondary outcomes within each symptom cluster. This less conservative approach is motivated by viewing the study design as a separate trial for each symptom cluster population. Of note, a finding of benefit in a particular symptom cluster population will only pertain to that symptom cluster population and will not be extrapolated to other symptom cluster populations.

10.5.1 PRIMARY EFFICACY ANALYSIS

Each of the three Symptom Cluster-specific primary endpoints is binary.

- For cognitive dysfunction, the primary endpoint is improvement of at least 5 T-score points on the PROMIS-cognitive 8a as measured at Day 90 compared to baseline. The proposed difference of 5 T-score points is derived from a body of literature that has specifically evaluated the minimally important difference using distribution- and anchor-based approaches across a number of disease processes (e.g., cancer, stroke, low back pain, osteoarthritis) and PROMIS measures (e.g., pain intensity, depression, physical functioning, anxiety, fatigue, etc.). These varied populations consistently report a minimally important difference of 2 to 6.[31-33] Moreover, a value of 5 represents one-half the standard deviation for PROMIS measures in the US general population and is recommended as a starting point when no empirical data are available.[34,35]
- For autonomic dysfunction, the primary endpoint is improvement as defined by a ≥ 1-point decrease in the OHQ question 1 at Day 90 compared to baseline. At least 1-point change on question 1 has been determined to be clinically meaningful and resulted in previous updates to the FDA drug label for orthostatic hypotension associated with Parkinson's disease.

- For exercise intolerance, the primary endpoint is improvement in PEM, defined as having no symptoms of moderate or greater severity with 50% or more frequency as determined by the DSQ-PEM short form at Day 90.

A separate pairwise comparison between intervention and control will be performed for each combination of study appendix, dose level, and symptom cluster. Analysis will target the intention-to-treat between-group difference in marginal probabilities of improvement, defined as

$$\Delta_{\mathrm{ITT}} = P(Y = 1|A = 1) - P(Y = 1|A = 0),$$

where Y denotes the primary outcome (1 = improvement, 0 = no improvement) and A denotes randomization assignment (1 = active treatment, 0 = control).

To improve precision, the estimate of Δ_{ITT} may be adjusted for a small number of pre-specified baseline covariates using the method of Ge et al. 2011 or similar (see SAP for details).[36] Covariates will be selected based on having a suspected strong association with primary outcomes and high likelihood of complete data capture. Inference for Δ_{ITT} will be based on Wald-type test statistics and associated confidence intervals.

Significance levels for the primary analysis will be determined by the multiplicity procedure described in above Section 10.5. Briefly, alpha will initially be equally allocated across symptom clusters (0.0166, 0.0166, 0.0166), with a total type-I error probability of 0.05. If the primary outcome comparison exceeds the critical threshold for some but not all symptom clusters, the procedure will allow non-significant comparisons to be re-tested using an updated, less stringent critical threshold.

The primary endpoints for the cognitive and autonomic symptom clusters were created by dichotomizing an underlying quantitative PRO outcome. An exploratory analysis of these outcomes will also be performed using the underlying quantitative measurements. While exploratory, these continuous outcomes may be estimated with greater precision compared to binary outcomes and will provide important context for the overall results interpretation. Methods for exploratory endpoints are described below.

10.5.2 ANALYSIS OF SECONDARY ENDPOINTS

Secondary outcomes are binary and will be analyzed using methods identical to the primary outcomes.

Secondary endpoint:
Meeting pre-specified change from baseline to Day 90 on cluster-specific performance outcome (yes/no).

Secondary cluster-specific performance outcomes:
Cognitive dysfunction symptom cluster: neurocognitive battery
Autonomic dysfunction symptom cluster: active stand test
Exercise intolerance symptom cluster: endurance shuttle walk test (ESWT)

10.5.3 ANALYSIS OF SAFETY ENDPOINTS

Safety endpoints include the proportion of participants who experience individual SAEs and the proportion who experience any one or more SAEs. Each of these will be compared pairwise between

each active intervention dose level and control. Events of Special Interest (ESIs) will be summarized by study drug appendix and duration. Incidence of AEs/SAEs leading to discontinuation of study intervention will also be summarized.

10.5.4 ANALYSIS OF EXPLORATORY ENDPOINTS

Exploratory endpoints that are binary will be analyzed using methods identical to the primary outcomes. Exploratory endpoints that are continuous will be analyzed via ANCOVA/regression with adjustment for pre-specified baseline covariates and with treatment effects expressed as differences in means. Covariates in each model will include an indicator of intervention group, the baseline value corresponding to the secondary outcome (e.g., adjust for baseline ESWT when analyzing End of Study ESWT as secondary outcome), and other pre-specified baseline factors.

10.5.5 EXPLORATORY LONGITUDINAL ANALYSES

Active intervention group comparisons of binary and continuous secondary outcomes will be assessed at multiple time points simultaneously by fitting longitudinal logistic (binary outcomes) and linear (continuous outcomes) regression models. Either hierarchical modeling with participant-specific random effects or Generalized Estimating Equations (GEE) methodology will be used to account for statistical dependence between repeated outcomes measured on the same participant over time.

Exploratory analysis will include analysis of symptom burden, PROs, exploratory performance measures at select sites, and healthcare utilization. Biomarkers will be explored to investigate mechanisms of potential intervention efficacy and to determine associated with PASC symptom clusters and intervention response. Symptom burden analysis will be presented with consideration to subgroups by baseline symptoms, racial and ethnic subgroup, sex, acute COVID-19 severity, and timing after acute infection. Refer to the SAP for details.

10.5.6 EXPLORATORY BAYESIAN ANALYSES

To enhance interpretation, a subset of primary, secondary, and exploratory outcomes described above will be re-analyzed in a Bayesian statistical framework. The Bayesian statistical paradigm is an attractive framework for exploratory analyses because it provides a direct quantification of the likelihood of various hypotheses that are relevant when considering whether and how to design a subsequent phase 3 evaluation. Traditional frequentist analyses assess evidence indirectly by positing a null hypothesis and calculating the probability of observing data at least as extreme as what was observed if the null hypothesis was true. Such an approach is indirect because it doesn't directly quantify the likelihood that a hypothesis is true or the likelihood that the trial will be successful. In contrast, Bayesian analysis can answer relevant questions exactly as they are posed. In particular, it can calculate the likelihood that a treatment is sufficiently effective to merit studying in a confirmatory phase 3 study. The output of a Bayesian analysis is a posterior probability distribution describing the relative likelihood of different numerical estimates of unknown quantities. This distribution can be used to determine the post-trial probability of a clinically important treatment effect in light of the study data. An additional advantage of Bayesian analysis is the ability to incorporate external information in the form of a prior distribution. For example, the prior may be formulated to reflect information from other RECOVER and non-RECOVER trials.

10.5.7 ANALYSIS OF TREATMENT EFFECT HETEROGENEITY

In addition to estimating the overall difference in outcome between intervention groups, exploratory analyses will assess whether and how the treatment effect differs according to a set of prospectively defined participant baseline factors, most importantly, vaccination status, duration of PASC symptoms, and selected virologic and/or inflammatory biomarkers (e.g., positive assay for antigenemia at baseline for antiviral agents). These analyses will be performed one-at-a-time for each subgroup variable using logistic regression for binary outcomes and linear regression for continuous outcomes. A treatment-by-covariate interaction test will be used to assess whether variation in the estimated treatment effect across levels of the covariate is consistent with chance.

As a further exploratory analysis, a Bayesian multivariable modeling strategy will be used to estimate the treatment effect for selected outcomes as a function of a small number of prospectively defined baseline covariates (e.g., duration of treatment, selected biomarkers). The analysis will improve on conventional subgroup analyses by analyzing multiple subgroup factors jointly in a single multivariable model and providing treatment effect estimates tailored to a specific combination of multiple relevant covariates. The analysis will also permit estimating treatment effects as a function of dose level in sub-studies investigating multiple intervention dose levels. The Bayesian prior distribution will place low prior probability on the hypothesis of extreme treatment effect variation across subgroups (i.e., a shrinkage prior) and will thereby reduce the probability of obtaining highly noisy and extreme estimates of treatment effect heterogeneity. Before developing these models, Monte Carlo simulations based on blinded RECOVER data will be used to shed light on the maximum feasible number of model covariates and will reduce the number of candidate covariates ahead of time if simulation results suggest a high potential for noisy estimates or false discoveries.

10.5.8 MISSING DATA

A particularly strong effort will be made to prevent participant's loss to follow-up and missing outcome data. However, in case of missing outcome data, patterns of missingness between intervention groups will be examined and compared to baseline characteristics and outcomes available in the missing and not missing groups to evaluate any systematic differences. The analysis strategy for the primary outcome analysis will be a covariate-adjusted logistic regression combined with direct standardization.[36,37] If outcome data are missing, participants with non-missing outcomes will be weighted to resemble the overall mITT population. Estimates will be unbiased under the assumption that outcome data are missing at random conditional on baseline covariates appearing in the weighting procedure. Sensitivity analyses will be performed under a range of missing data assumptions and may include multiple imputation, weighting, and likelihood-based methods. See SAP for details.

10.5.9 PLANNED INTERIM ANALYSIS

Interim examination of clinical endpoints and key safety events will be performed at regular intervals during the course of each sub-study/appendix. An independent, NIH-appointed, DSMB will monitor participant safety and review participant enrollment and performance of the trial. The primary objective of these interim analyses will be to ensure the safety of the participants enrolled in the trial and evaluate the accumulating endpoint data by treatment group. In addition, interim monitoring will involve a review of participant recruitment, compliance with the study protocol, status of data collection, and other factors which reflect the overall progress and integrity of the study.

There are no planned early stopping rules for efficacy in this protocol. Because PASC presentations and outcomes are highly varied, an important study objective is to estimate the effect of treatment on a wide range of participant-relevant outcomes. If the study were to be stopped early with less than the full sample size, it would decrease precision and reduce the study's ability to characterize treatment risks and benefits based on important secondary effectiveness and safety outcomes. It would also limit the collection of data that are critical for planning future trials in similar patient populations.

Early stopping for rules for futility may be incorporated on a sub-study/appendix specific basis. Such rules will be documented in the sub-study appendices.

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

11.1.1 INFORMED CONSENT PROCESS

11.1.1.1 INSTITUTIONAL REVIEW BOARD (IRB)

The protocol, informed consent form(s) [ICF(s)], recruitment materials, and all participant materials will be submitted to the Institutional Review Board(s) [IRB(s)] of record for review and approval. This approval must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB(s) before being implemented in the study. All changes to the consent form will also be IRB-approved and a determination will be made regarding whether previously consented participants need to be re-consented.

11.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

All consenting will occur either via an electronic consent process or a paper process. Consent forms describing in detail the study intervention/control, study procedures, and risks will be given to the participant and documentation of informed consent is required prior to starting study procedures. Informed consent is a process that is initiated prior to the individual’s agreement to participate in the study and continues throughout the individual’s study participation. A description of risks and possible benefits of participation will be provided to the participants. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The participant will be provided contact information in the event they have questions about study participation. This will allow them to communicate with the investigators (or their delegate), for further explanation of the research study and to answer any questions that may arise, as necessary. Participants will have the opportunity to carefully review the consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study and think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be provided to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The study team will distinguish between the desire to discontinue study intervention(s) and the desire to withdraw consent for study follow-up.

11.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the sponsor to study participants, site investigators, the central IRB, and the US FDA. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

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

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

11.1.3 CONFIDENTIALITY AND PRIVACY

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the CT-DCC. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected. At the end of the study, all study-related data storage systems will be archived according to local processes.

11.1.4 KEY ROLES AND STUDY GOVERNANCE

The RECOVER program is overseen by the RECOVER Executive Committee, the NIH RECOVER Program, and the RECOVER Senior Oversight Committee Co-Chairs. The RECOVER program also includes a Clinical Trial Steering Committee, which is a multi-stakeholder committee that oversees the study and includes patients, the CT-DCC, the NIH, the FDA, and academic and subject matter experts.

The CT-DCC is overseen by a Principal Investigator. The CT-DCC is responsible for study coordination, site management, communication, financial administration, treatment allocations, receipt and processing of data, quality control programs, and statistical analysis and reporting.

The DSMB will oversee the safety and welfare of trial participants as well as provide recommendations for continuation, discontinuation, or revision of the trial.

11.1.5 DATA AND SAFETY MONITORING BOARD

Safety oversight will be under the direction of the RECOVER DSMB composed of individuals with the appropriate expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semi-annually to approve protocols, assess safety and efficacy data, and at appropriate intervals to meet requirements for the Interim Analyses on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the NIH.

11.1.6 CLINICAL MONITORING

This study will employ a centralized risk-based approach to monitoring with routine and periodic review of site-submitted data to review the informed consent process, select eligibility criteria, medical history, identify and follow-up on missing data, inconsistent data, data outliers, etc. and ensure completion of administrative and regulatory processes. The study team will facilitate regular communication through training sessions, teleconferences, videoconferencing, email, etc. Using quality-by-design principles, steps will be taken at the study design stage to foresee and limit significant problems that might occur during the study conduct. Follow-up from the sites is expected to keep participants engaged.

11.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

The study team will work in tandem to ensure that the data collected in this study are as complete and correct as possible. A four-step, multi-functional approach to quality control will be implemented:

- Training: Prior to the start of enrollment, the clinician investigators and key study personnel at each site will be trained with the clinical protocol and data collection procedures, including how to use the EDC system. Follow-up training and training for new study personnel or new versions of the protocol will be conducted as needed.

- Monitoring: The RECOVER CT-DCC will ensure that data collection is handled properly, will provide in-service training, and will address questions from site investigators and coordinators. Electronic review of data quality and completeness will occur on a regular and ongoing basis. Any issues will be addressed.

- Managing data: After the data have been transferred for statistical summarization, data description, and data analysis, further crosschecking of the data will be performed with discrepant observations being flagged and appropriately resolved through a data query system.

- Reviewing data: Data regarding events of interest will be reviewed to ensure appropriate documents are collected for DSMB review. The CT-DCC will monitor study data and contact site study teams when events comprising the primary endpoint are not complete.

11.1.8 DATA HANDLING AND RECORD KEEPING

11.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Streamlining 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. Data may be collected by electronic methods, supplemented by telephone or videophone follow-up, and from the electronic health record.

Data will be collected directly from participants using REDCap through text messaging or email with a survey link, or phone call as back up. The process for using text messaging and email is Health Insurance Portability and Accountability Act (HIPAA) compliant.

Site personnel or participants will enter study data into a secure online database. Data will be maintained in a secure online database until the time of study publication. At the time of publication, the CT-DCC will generate a de-identified version of the database for archiving (see Section 11.1.10). All source documents at the sites should be completed in a neat, legible manner to ensure accurate interpretation of data.

11.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of six years after the study has ended. However, if required by local regulations or the US FDA, these documents should be retained for a longer period. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

11.1.9 PROTOCOL DEVIATIONS

A protocol deviation is defined as non-compliance with the clinical study protocol or GCP requirements. The non-compliance may be on the part of the participant, site investigator, or the site staff.

A major protocol deviation is a significant divergence from the protocol that may have significant effect on the participant's safety, rights, or welfare and/or on the integrity of the study data. Major protocol deviations must be sent to the study IRB and local IRB per their guidelines, recorded in source documents, and reported to the coordinating center. All protocol deviations will be documented. For this study, any missed or delayed survey completion will not be considered a major protocol deviation, unless it is a study procedure that is required for the primary endpoint.

11.1.10 PUBLICATION AND DATA SHARING POLICY

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

11.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.2 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase
ANCOVA	Analysis of Covariance
AST	Aspartate Aminotransferase
BP	Blood Pressure
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus Disease of 2019
CRF	Case Report Form
CTCSQ	Cluster Targeted COVID-19 Symptom Questions
CT-DCC	Clinical Trial – Data Coordinating Center
DCRI	Duke Clinical Research Institute
DILI	Drug-Induced Liver Injury
DSMB	Data Safety Monitoring Board
DSQ - PEM	DePaul Symptom Questionnaire Post Exertional Malaise
DUA	Data Use Agreement
ECOG 2	Everyday Cognition 2
eCRF	Electronic Case Report Forms
EOD	End of Dosing
ESI	Events of Special Interest
ESWT	Endurance Shuttle Walk Test
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HRQOL	Health-Related Quality of Life
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISWT	Incremental Shuttle Walk Test
LFT	Liver Function Test
mITT	Modified Intention-To-Treat
MOP	Manual of Procedures
NCT	National Clinical Trial

NIH	National Institutes of Health
OHQ	Orthostatic Hypotension Questionnaire
OIDAS	Orthostatic Intolerance Daily Activity Scale
OISA	Orthostatic Intolerance Symptom Assessment
PASC	Post-acute Sequelae of SARS-CoV-2 Infection
PCR	Polymerase Chain Reaction
PEM	Post-Exertional Malaise
PHI	Personal Health Information
PRO	Patient Reported Outcome
RAT	Rapid Antigen Test
RECOVER	Researching COVID to Enhance Recovery
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SUSAR	Suspected Unexpected Serious Adverse Reaction
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
US	United States
VO2 max	Maximum Rate of Oxygen Consumption

11.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change
1.0	10OCT2022	None, original protocol
2.0	09MAR2023	• Updated study description, background, and rationale; • Moved biomarker objective to exploratory, added an additional biomarker exploratory objective (exploratory objective #6), and added more details around exploratory biomarker outcome measures; • Adjusted sample size from 1700 to 900; • Added reference to and description of potential phase 2 and phase 3 study design within the appendices; • Extended follow-up to 6 months; • Study Schema updated to align with study design changes; • Symptom cluster assignment flow updated; • Limits were placed on the number of participants with suspected and probable COVID-19 on or after May 1, 2021; • Two moderate symptoms from the same Symptom Cluster or one severe symptom required for inclusion; • End of Study has been extended to 183 days from the original 90 days; • PROMIS 29+2, PASC Symptom Questionnaire, and PRO Symptom Cluster Assessment(s) will be done weekly as opposed to every 2 weeks;

		• Stool collection added; • Updated clinical laboratory assessments; • Optional blood collection/nasal swab visits added to Days 45, 60, and 120; details on biospecimen collection added; • Added ECOG 2 to the cognitive dysfunction PRO assessment; • Non-serious AEs that result in study drug discontinuation will now be reposted separately as an AE; • Statistical considerations updated to align with study design changes; • PAXLOVID dosing modified to 15 days of active DOI (nirmatrelvir/ritonavir) with 10 days of control (ritonavir), 25 days of active DOI, or 25 days of control (1:1:1); • Dose-related exploratory objectives have been added to Appendix A (PAXLOVID Phase 2); • Thyroid function added as an Event of Special Interest to Appendix A (PAXLOVID Phase 2); and • Other administrative changes throughout.
3.0	18MAY2023	• Updated schema to align with changes to study procedures; • Clarified study endpoints, referred to stats section for details; • Updated exploratory objectives; • Added the collection of non-serious AEs to the protocol, non-serious AEs will be assessed from start of study drug administration to the EOD for select appendices; • Participants will have 3 days to complete remote weekly assessments; • DSQ-PEM completed after performance measures will occur at 1 (+ 1 day) after performance measure; • Updated statistical considerations; • Removed exclusion #9 because it duplicates exclusion #8; • Added a row for "Healthcare Utilization" to the Schedule of study procedures; • Clarified that stool collection will be processed at a central lab; • Added that peripheral blood mononuclear cells will be collected at select sites; • Added additional appendix-level study assessments to Appendix A; • Updated drug storage specifications for Appendix A; and • Other administrative changes throughout.

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13 APPENDIX A (PAXLOVID PHASE 2)

This appendix describes a prospective, multi-center, double-blind, randomized controlled phase 2 trial evaluating nirmatrelvir/ritonavir (PAXLOVID) in two dosing durations for PASC. Within this study, participants will be randomized 1:1:1 to 15 days of active intervention (nirmatrelvir/ritonavir) with 10 days of control (placebo/ritonavir), 25 days of active intervention, or 25 days of control, within three symptom clusters. The intervention will be administered in two kits (15 days followed by 10 days). For this appendix, the midpoint of drug dosing is approximately 13 days; the Drug Dosing Midpoint Clinic Visit will occur at Day 15 (-5 days).

13.1 RISK ASSESSMENT

Serious and unexpected AEs may occur that have not been previously reported with PAXLOVID use.[38] Previously reported AEs were identified in the EPIC-HR study, which was a phase 2/3 randomized, controlled trial in non-hospitalized adults with a laboratory confirmed diagnosis of SARS-CoV-2.[39] EPIC-HR AEs included (incidence ≥ 1% and ≥ 5 subject difference) dysgeusia, diarrhea, hypertension, and myalgia. Following Emergency Use Authorization (EUA) use of PAXLOVID, hypersensitivity reactions, abdominal pain, nausea, and malaise have been reported. Otherwise, any reported AEs in humans are related either to nirmatrelvir or ritonavir use. For example, in patients receiving ritonavir, hepatic transaminase elevations, clinical hepatitis, and jaundice have occurred.

No PAXLOVID human data are currently available to evaluate for a drug-associated risk of major birth defects, miscarriage, adverse maternal or fetal outcomes, or lactation effects. However, one study is currently recruiting pregnant women with mild or moderate COVID-19 (NCT05386472), and another study will be examining PAXLOVID in healthy lactating women (NCT05441215). Pre-clinical data indicate that there are no clinically relevant risks associated with PAXLOVID administration during pregnancy and in males and females of reproductive age.[40] Published observational studies on ritonavir use in pregnant women have not identified an increased risk of major birth defects. Published studies with ritonavir are insufficient to identify a drug-associated risk of miscarriage. The benefit of taking PAXLOVID during pregnancy may be greater than the risk from the treatment; however, the risk/benefit of treatment for PASC during pregnancy has not been established.

13.2 ADDITIONAL APPENDIX-LEVEL EXPLORATORY OBJECTIVE(S)

OBJECTIVE(S)	OUTCOME MEASURES	ENDPOINTS
Exploratory		
Describe the effect of dose on efficacy of intervention within each PASC symptom cluster	**Cognitive dysfunction symptom cluster:** PROMIS – cognitive 8a **Autonomic dysfunction symptom cluster:** OHQ **Exercise intolerance symptom cluster:** DSQ-PEM	**Cognitive dysfunction symptom cluster:** Participants meeting pre-specified change from baseline to Day 90 on PROMIS - cognitive 8a T-score **Autonomic dysfunction symptom cluster:** Participants meeting pre-specified change from baseline to Day 90 on the OHQ

OBJECTIVE(S)	OUTCOME MEASURES	ENDPOINTS
		Exercise intolerance symptom cluster: Participants meeting pre-specified change from baseline to Day 90 on the DSQ-PEM
Characterize the effect of dose on safety and tolerability of intervention in PASC	• Serious adverse events (SAEs) • Events of Special Interest (ESIs) • Adverse events (AEs) • Number of missed doses (adherence)	• Occurrence of individual SAEs and AEs • Occurrence of any one or more SAEs and AEs • Occurrence of AEs and SAEs leading to discontinuation • Occurrence and duration of ESIs Number of missed doses (adherence)

Refer to the SAP for analysis details.

13.3 ADDITIONAL APPENDIX-LEVEL EXCLUSION CRITERIA

1. Known pregnancy[1]
2. Active or expected breastfeeding during the study
3. Known eGFR < 30 mL/min
4. Known severe hepatic impairment (Child-Pugh Class C)
5. Current use of drugs highly dependent on CYP3A for clearance[2] and for which elevated concentrations are associated with serious and/or life-threatening reactions and which cannot be interrupted during the time of study administration and within seven days before and after study drug administration
6. Current use of potent CYP3A inducers[2] where significantly reduced nirmatrelvir or ritonavir plasma concentrations may be associated with the potential for loss of virologic response and possible resistance

[1]*A pregnancy test must be performed at the Baseline Visit for participants who are capable of becoming pregnant.*
[2]*A guide of drugs that may be contraindicated are listed in Section 4 CONTRAINDICATIONS of the Full Prescribing Information of the EUA for PAXLOVID.*

13.4 ADDITIONAL APPENDIX-LEVEL STUDY ASSESSMENTS

Safety Labs for appendix A are drawn in addition to platform protocol labs at the following time points and are run locally at the site. All non-serious AEs will be collected and reported from start of study drug dosing to end of study drug administration for this appendix at all timepoints that designate collection of non-serious AEs in Table 2.

Baseline Visit

- Pregnancy test (for participants who are capable of becoming pregnant)
- Thyroid stimulating hormone (TSH)

- Total and free triiodothyronine (T3, total and free)
- Total and free thyroxine (T4, total and free)

Drug Dosing Midpoint Visit

- Thyroid stimulating hormone (TSH)
- Total and free triiodothyronine (T3, total and free)
- Total and free thyroxine (T4, total and free)
- Comprehensive metabolic panel

End of Dosing Visit

- Thyroid stimulating hormone (TSH)
- Total and free triiodothyronine (T3, total and free)
- Total and free thyroxine (T4, total and free)
- Comprehensive metabolic panel

90 Day Visit

- Thyroid stimulating hormone (TSH)
- Total and free triiodothyronine (T3, total and free)
- Total and free thyroxine (T4, total and free)
- Comprehensive metabolic panel

13.5 PAXLOVID INFORMATION

In December 2021, the US FDA issued an EUA for use of the unapproved PAXLOVID, which includes nirmatrelvir and ritonavir for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (12 years of age and older weighing at least 40 kg) who test positive for SARS-CoV-2 and are at high risk for progression to severe COVID-19.[38] Authorized dosing includes 300 mg nirmatrelvir with 100 mg ritonavir twice daily for five days.

PAXLOVID is nirmatrelvir tablets co-packaged with ritonavir tablets. Nirmatrelvir is a SARS-CoV-2 main protease inhibitor. Ritonavir is an HIV-1 protease inhibitor and CYP3A inhibitor. Since CYP3A metabolizes nirmatrelvir, ritonavir produces higher systemic concentrations and longer half-life of nirmatrelvir.

13.5.1 PRECAUTIONS

The healthcare provider should consult other appropriate resources such as the prescribing information for the interacting drug for comprehensive information on dosing or monitoring with concomitant use of a strong CYP3A inhibitor such as ritonavir. No dosage adjustment is required when co-administered with other products containing ritonavir or cobicistat. Participants on ritonavir- or cobicistat-containing Human Immunodeficiency Virus (HIV) or Hepatitis C viral regimens should continue their treatment as indicated.

Initiation of medications that inhibit or induce CYP3A may increase or decrease concentrations of PAXLOVID, respectively. These interactions may lead to:

- Clinically significant adverse reactions, potentially leading to severe, life-threatening, or fatal events from greater exposures of concomitant medications

- Clinically significant adverse reactions from greater exposures of PAXLOVID
- Loss of therapeutic effect of PAXLOVID and possible development of viral resistance

13.5.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

PAXLOVID is a combination of nirmatrelvir and ritonavir tablets.

Formulation:

- Nirmatrelvir is available as immediate-release, film-coated tablets. Each tablet contains 150 mg nirmatrelvir with the following inactive ingredients: colloidal silicon dioxide, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and sodium stearyl fumarate. The following are the ingredients in the film coating: hydroxy propyl methylcellulose, iron oxide red, polyethylene glycol, and titanium dioxide.
- Ritonavir is available as film-coated tablets. Each tablet contains 100 mg ritonavir with the following inactive ingredients: anhydrous dibasic calcium phosphate, colloidal silicon dioxide, copovidone, sodium stearyl fumarate, and sorbitan monolaurate. The film coating may include the following ingredients: colloidal anhydrous silica, colloidal silicon dioxide, hydroxypropyl cellulose, hypromellose, polyethylene glycol, polysorbate 80, talc, and titanium dioxide.

Appearance:

- Nirmatrelvir is supplied as oval, pink, film-coated tablets.
- Ritonavir is supplied as ovaloid, white, film-coated tablets.

13.5.3 DRUG DISPENSING, STORAGE, AND STABILITY

Store at 15°C to 30°C (59°F to 86°F). Participants will receive two kits. Kit 1 will be provided at the Baseline Visit to cover days 1 to 15. Kit 2 will be provided at the midpoint dosing visit to cover days 16 to 25.

13.5.4 DOSING AND ADMINISTRATION

PAXLOVID dosage is 300 mg nirmatrelvir (two 150 mg tablets) with 100 mg ritonavir (one 100 mg tablet). All three tablets (two of nirmatrelvir and one of ritonavir) must be taken together and no more than 15 minutes apart. For this appendix, all three tablets are taken together twice daily by mouth for 25 days. PAXLOVID tablets can be taken with or without food, however, taking with food may improve tolerability. PAXLOVID tablets should be swallowed whole, not chewed, broken, or crushed.

All participants will undergo dosing for 25 days. Those randomized to PAXLOVID for 15 days will receive 15 days of active intervention (nirmatrelvir/ritonavir) and 10 days of control (placebo/ritonavir). Those randomized to PAXLOVID for 25 days will receive 25 days of active intervention.

Each participant will receive two kits that include a combination of blister cards and bottles. All participants will be instructed to take two tablets from the blister cavities and one tablet from the bottle for each dose. Refer to the MOP for details.

A dose reduction is necessary for participants with moderate renal impairment (eGFR ≥ 30 to < 60 mL/min): reduce to 150 mg nirmatrelvir (one 150 mg tablet) with 100 mg ritonavir (one 100 mg tablet),

with both tablets taken together twice daily for the dosing period. No dose reduction is needed in participants with mild renal impairment (eGFR ≥ 60 to < 90 mL/min).

If the participant misses a dose of PAXLOVID within 8 hours of the time it is usually taken, the participant should take it as soon as possible and resume the normal dosing schedule. If the participant misses a dose by more than 8 hours, the participant should not take the missed dose but instead take the next dose at the regularly scheduled time. The participant should not double the dose to make up for a missed dose.

For this study, any dose of nirmatrelvir greater than 900 mg (6 nirmatrelvir tablets) or ritonavir greater than 300 mg (3 ritonavir tablets) within a 24-hour time period will be considered an overdose. There is no specific treatment for an overdose. Overdose is reportable to Pfizer Safety by DCRI Safety Surveillance **only when associated with an SAE**. In the event of an overdose, the investigator should:

1. Closely monitor the participant for any AEs/SAEs and laboratory abnormalities for at least 5 half-lives or 28 calendar days after the overdose of study intervention (whichever is longer).
2. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.
3. If there is an associated SAE, the SAE is reported within the CRF within 24 hours of site awareness.

Decisions regarding dose interruptions or modifications will be made by the site investigator based on the clinical evaluation of the participant.

13.5.5 RATIONALE FOR SELECTION OF DOSE

Pre-clinical studies:
Nirmatrelvir has been characterized in nonclinical safety pharmacology and repeat-dose toxicity studies in the rat and non-human primate. Ritonavir was not included in pre-clinical safety studies for multiple reasons, as noted by Sathish et al.[41]:

- ritonavir is a marketed drug with a well-characterized pre-clinical and clinical safety profile, and no overlapping or additive toxicities between nirmatrelvir and ritonavir are expected;
- the exposure-enhancing effects of ritonavir seen in humans do not translate completely in animals;
- high exposures of nirmatrelvir in pre-clinical studies were achieved through use of solvate and formulations approaches.

High-dose nirmatrelvir administration (75 mg/kg per dose twice daily, by mouth) over a 5-day treatment period in cynomolgus monkeys produced cardiovascular effects limited to transient increases in BP and decreases in heart rate. Cardiovascular effects were not observed in low dose (20 mg/kg per dose twice daily, by mouth). Additionally, no adverse findings were observed in repeat dose toxicity studies up to 29 days in rats (up to 1,000 mg/kg daily, by mouth) or monkeys (up to 600 mg/kg daily, by mouth).

Clinical studies:
A first-in-human study (NCT04756531) assessed nirmatrelvir safety, tolerability, and pharmacokinetics following single-ascending doses including food effect, multiple-ascending doses, and at supra-therapeutic exposure in healthy 18 to 60-year-old patients.[42] Participants who received multiple-ascending doses were randomly assigned to control or escalating nirmatrelvir dose levels (75, 250, and 500 mg), all administered twice daily as a suspension for 10 days with ritonavir 100 mg, under fasting conditions. At all dose levels, nirmatrelvir plasma concentrations reached steady state by Day 2 and

remained steady through Day 10. Across all multiple-ascending dose groups and control, treatment-related AEs included mild dysgeusia (n = 3) and mild increases in blood thyroid stimulating hormone (n = 5). No severe AEs or SAEs were reported.

Moreover, a supra-therapeutic oral dose of nirmatrelvir as a 2,250-mg suspension (dosed as 3 split doses of 750 mg administered at 0, 2, and 4 hours) enhanced with 100 mg ritonavir increased the mean plasma concentration ~ 5 times higher than that of the 500 mg nirmatrelvir multiple-ascending dose cohort on Day 1. Even with this high concentration, only one case of mild nausea in the treatment group was observed. No severe AEs or SAEs were reported.

In clinical studies in participants at high risk of progression to severe COVID-19 illness, PAXLOVID, as compared to placebo, was associated with an approximately 0.9 log10 copies/mL greater decline in viral RNA levels in nasopharyngeal samples through Day 5,[38] which is the approved dosing duration for acute COVID-19.[38]

To date the appropriate duration of therapy for PASC remains unknown. This study will test two dosing durations, 15 days and 25 days, which have been chosen to enable evaluation of dose response of prolonged therapy within the range of dosing durations previously used in select clinical practice (e.g., immunosuppression).

No randomized clinical trial has been performed to study PAXLOVID in patients with PASC. Case studies of patients with PASC taking PAXLOVID over 5 days show equivocal improvements in symptom resolution.[43]

The planned dosing duration in this study (15 and 25 days) is nearly three and five times that typically administered and currently under EUA for administration to those with acute SARS-CoV-2 infection for prevention of severe disease. Previous clinical trials have confirmed the safety of PAXLOVID treatment up to a duration 10 days and data suggests similar safety/tolerability of 5- and 10-day treatment durations. In addition, prolonged durations of PAXLOVID (up to 20 days) have been used in heavily immunosuppressed patients with persistent SARS-CoV-2 infection,[44] and for other disease processes where viral persistence and reactivation are of concern (e.g., herpes simplex virus, especially neonatal herpes simplex virus), prolonged durations of antivirals (sometimes up to 1 year) are standard. Moreover, more prolonged durations might increase the probability of viral eradication. Viral eradication is the mechanism by which antivirals are believed to potentially mitigate PASC.

13.6 CONTROL INFORMATION

In order to maintain blinding with the distinct taste of ritonavir, the control for this appendix will be the combination of ritonavir and placebo. Data obtained from a mouse study show no evidence of an antiviral effect of ritonavir alone (see Figure 5). As such, ritonavir with placebo is expected to be an appropriate control for the PAXLOVID appendix.

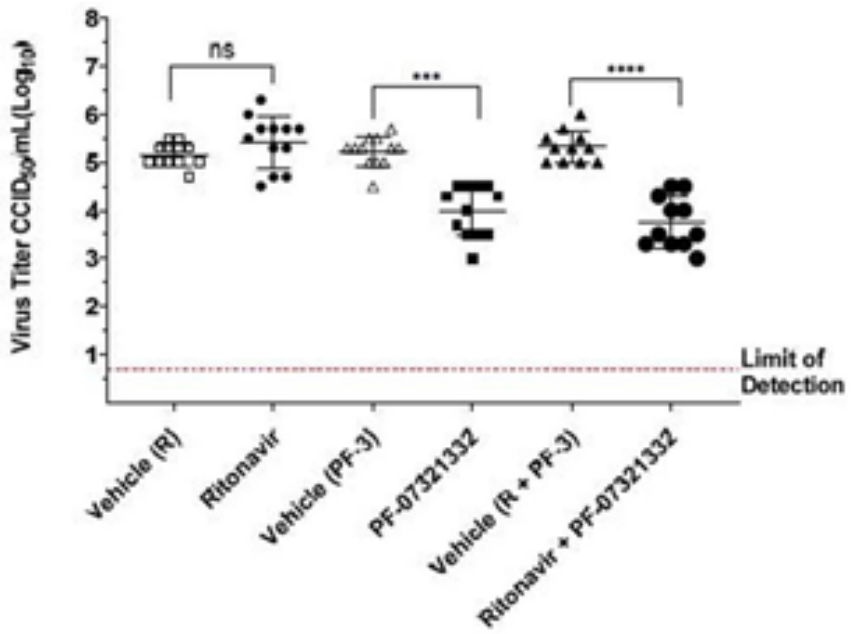

Figure 5. Effect of PAXLOVID (PF-07321332) on lung viral titers following oral administration in BALB/c mice infected with SARS-COV-2-MA10

Twice daily oral administration of PF-07321332 alone at 300 mg/kg or at 300 mg/kg in the presence of 50 mg/kg ritonavir reduced SARS-CoC-2 lung viral titer in mice. Mice (N=12) were inoculated with MA-SARS-CoV-2. ***p<0.0005; ****p<0.0001; PF-3 = PF-07321332; R = ritonavir.

13.6.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The control formulation includes two placebo tablets and one ritonavir tablet. The following ingredients are in a 210 mg placebo tablet: microcrystalline cellulose, NF (MC-102); pregelatinized starch, NF (Starch 1500); croscarmellose sodium, NF (Vivasol, GF Grade); colloidal silicon dioxide, NF (Aerosil 200); and magnesium stearate, NF (2257).

Control appearance will match that of all three PAXLOVID tablets (2 nirmatrelvir tablets and 1 ritonavir tablet):

- The placebo for the 150 mg nirmatrelvir tablet is supplied as an oval, pink, film-coated tablets.
- Ritonavir is supplied as ovaloid, white, film-coated tablets.

The control packaging matches the packaging described in Section 13.5.2.

13.6.2 CONTROL DISPENSING, STORAGE, AND STABILITY

Store at 15°C to 30°C (59°F to 86°F).

13.6.3 DOSING AND ADMINISTRATION

Control dosing and administration will occur according to Section 13.5.4 in order to maintain blinding.

13.7 EVENTS OF SPECIAL INTEREST

Alteration in Thyroid Function

Alterations in thyroid function have been observed with patients who take PAXLOVID for longer than 10 days. Therefore, all participants in the study will undergo evaluation of thyroid stimulating hormone (TSH), total and free triiodothyronine (T3), and total and free thyroxine (T4) at baseline, drug dosing midpoint clinic visit, EOD, and 90 days.

Drug-Induced Liver Injury (DILI)

Humans exposed to a drug who show no sign of liver injury (as determined by elevations in transaminases) are termed "tolerators," while those who show transient liver injury but adapt are termed "adaptors." In some participants, transaminase elevations are a harbinger of a more serious potential outcome. These participants fail to adapt and therefore are "susceptible" to progressive and serious liver injury, commonly referred to as DILI. Participants who experience a transaminase elevation above 3 × upper limit of normal (ULN) should be monitored more frequently to determine if they are "adaptors" or are "susceptible."

Liver function tests (LFTs) are not required as a routine safety monitoring procedure in this study. However, should an investigator deem it necessary to assess LFTs because a participant presents with clinical signs/symptoms, such LFT results should be managed and followed as described below.

In the majority of DILI cases, elevations in aspartate aminotransferase (AST) and/or alanine transaminase (ALT) precede T bili (bilirubin) elevations (> 2 × ULN) by several days or weeks. The increase in T bili typically occurs while AST/ALT is/are still elevated above 3 × ULN (i.e., AST/ALT and T bili values will be elevated within the same laboratory sample). In rare instances, by the time T bili elevations are detected, AST/ALT values might have decreased. This occurrence is still regarded as a potential DILI. Therefore, abnormal elevations in either AST **OR** ALT in addition to T bili that meet the criteria outlined below are considered potential DILI (assessed per Hy's law criteria) cases and should always be considered important medical events, even before all other possible causes of liver injury have been excluded.

The threshold of laboratory abnormalities for a potential DILI case depends on the participant's individual baseline values and underlying conditions. Participants who present with the following laboratory abnormalities should be evaluated further as potential DILI (Hy's law) cases to definitively determine the etiology of the abnormal laboratory values:

1. Participants with AST/ALT and T bili baseline values within the normal range who subsequently present with AST **OR** ALT values ≥ 3 × ULN **AND** a T bili value ≥ 2 × ULN with no evidence of hemolysis and an alkaline phosphatase value < 2 × ULN or not available.

2. For participants with baseline AST **OR** ALT **OR** T bili values above the ULN, the following threshold values are used in the definition mentioned above, as needed, depending on which values are above the ULN at baseline:

    a. Preexisting AST or ALT baseline values above the normal range: AST or ALT values ≥ 2 times the baseline values AND ≥ 3 × ULN; or ≥ 8 × ULN (whichever is smaller).

b. Preexisting values of T bili above the normal range: T bili level increased from baseline value by an amount of ≥ 1 × ULN **or** if the value reaches ≥ 3 × ULN (whichever is smaller).

Rises in AST/ALT and T bili separated by more than a few weeks should be assessed individually based on clinical judgment; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor.

The participant should return to the investigator site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history, and physical assessment.

In addition to repeating measurements of AST and ALT and T bili for suspected Hy's law cases, additional laboratory tests should include albumin, creatinine kinase, direct and indirect bilirubin, gamma-glutamyl transferase, prothrombin time test and INR (PT/INR), total bile acids, and alkaline phosphatase. Consideration should also be given to drawing a separate tube of clotted blood and an anticoagulated tube of blood for further testing, as needed, for further contemporaneous analyses at the time of the recognized initial abnormalities to determine etiology. A detailed history, including relevant information, such as review of ethanol, acetaminophen/paracetamol (either by itself or as a coformulated product in prescription or over-the-counter medications), recreational drug, or supplement (herbal) use and consumption, family history, sexual history, travel history, history of contact with a jaundiced person, surgery, blood transfusion, history of liver or allergic disease, and potential occupational exposure to chemicals, should be collected. Further testing for acute hepatitis A, B, C, D, and E infection, liver imaging (e.g., biliary tract), and collection of serum samples for acetaminophen/paracetamol drug and/or protein adduct levels may be warranted.

All cases demonstrated on repeat testing as meeting the laboratory criteria of AST/ALT and T bili elevation defined above should be considered potential DILI (Hy's law) cases if no other reason for the LFT abnormalities has yet been found. **Such potential DILI (Hy's law) cases are to be reported as SAEs, irrespective of availability of all the results of the investigations performed to determine etiology of the LFT abnormalities.**

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology.

13.8 APPENDIX HALTING RULES

Dosing and enrollment will be temporarily suspended pending review of the DSMB if three or more participants experience an SAE of the same type that is determined to be related to study intervention (s). The DSMB recommendations will be considered by the NIH and the study Principal Investigator prior to making any decisions regarding appendix continuation or discontinuation.