RECOVER-AUTONOMIC: A Platform Protocol for Evaluation of Interventions for Autonomic Dysfunction in Post-Acute Sequelae of SARS-CoV-2 Infection (PASC)

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1 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.

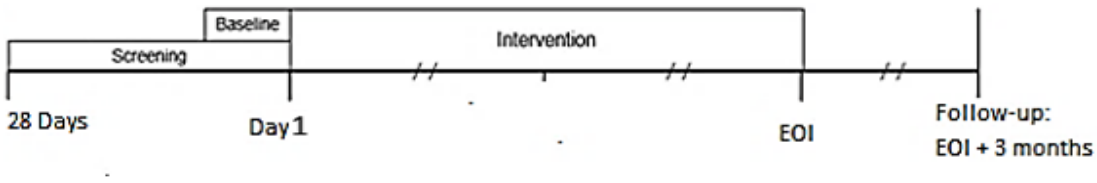
1.1 SYNOPSIS

Title:	RECOVER-AUTONOMIC: A Platform Protocol for Evaluation of Interventions for Autonomic Dysfunction 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 in community settings where it can be integrated into COVID-19 programs and subsequent treatment plans. This protocol is a prospective, multi-center, multi-arm, randomized, controlled platform trial evaluating various interventions for use in the treatment of autonomic dysfunction symptoms, including cardiovascular complications and postural orthostatic tachycardia syndrome (POTS), in PASC participants. The interventions tested will include non-pharmacologic care and pharmacologic therapies with study drugs. The hypothesis is that some of the autonomic dysfunction symptoms are immune-mediated, so immunotherapy and other applicable therapies will result in improvement in autonomic symptoms.
Objectives:	Primary: 1. Evaluate the effect of study intervention versus control on orthostatic intolerance

	Secondary: 2. Evaluate the effect of study intervention versus control on symptom-specific outcome measures 3. Describe the effect of study intervention versus control on performance-based and vital sign outcome measures 4. Evaluate the effect of study intervention versus control on quality of life 5. Compare the effect of study intervention versus control on specific tracked measurements 6. Characterize the safety and tolerability of study intervention for treatment of PASC Exploratory: 7. Evaluate the effect of study intervention versus control on autonomic function tests 8. Describe the effect of study intervention versus control on performance-based measures 9. Describe the effect of study intervention versus control on PASC symptoms 10. Evaluate the effect of study intervention versus control on relevant biomarkers
Study Population:	Each study appendix will enroll approximately 180-200 evaluable adult participants who experience autonomic dysfunction symptoms of PASC. This is expected to be sufficient to conclude whether there is meaningful evidence of intervention benefit. Overall enrollment will depend on the number of screen failures, the number of study drug appendices that are added, the ability to pool their control groups for analysis, and adjustments to sample size based on-study data. The goal is to have a diverse population, including the underserved communities and racial/ethnic populations frequently underrepresented in clinical research.
Phase:	Phase 2
Description of Sites/Facilities Enrolling Participants:	Participants will be recruited from various acute COVID-19 trials and existing RECOVER initiatives as well as other sites and research communities. Approximately up to 75 sites in the US will participate.
Description of Study Intervention:	Each study appendix describes a different intervention and control.
Participant Duration:	Duration of intervention will vary depending on the specific study appendix. Participants will have one follow-up visit approximately 3 months after the end of intervention.

1.2 SCHEMA

Figure 1. Platform Protocol Study Schema



1.3 KEY ROLES

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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 the novel coronavirus disease 2019 (COVID-19).[1-4] The personal and global impact of these long-term symptoms from SARS-CoV-2 infection can be debilitating, and the number of PASC 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 for autonomic symptoms in PASC. If successful, this trial will rapidly provide the foundational evidence to enable providers to identify and treat and ameliorate symptoms in PASC patients.

2.2 BACKGROUND

In 2019, 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, and other serious, life-threatening complications.

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 (PASC), also known as PASC, is a chronic condition present in up to 80% of infected, hospitalized patients and 40% to 70% of non-hospitalized patients.[1-4,6] PASC 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, 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 worsening a decade-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 3 predominant symptom clusters, including exercise intolerance, cognitive dysfunction, and autonomic dysfunction that are frequently reported but also of substantial importance to patients. In addition, data have identified substantial heterogeneity in symptomology and presentation, even among those within a specific symptom cluster; and the lack of concordance between objective findings and reported symptoms in many cases.[7,8]

Autonomic multisystem dysfunction including cardiovascular dysregulation, and postural tachycardia syndrome (POTS), are often seen in PASC patients. Postural orthostatic tachycardia syndrome (POTS) is an abnormal increase in heart rate (HR) that occurs when transitioning from lying down to standing up.

Among COVID-19 survivors, 9% to 16% experience POTS-like symptoms, such as tachycardia, orthostatic intolerance, fatigue, and cognitive impairment, and 2% to 14% develop POTS.[9]

Immune therapies, such as intravenous immunoglobulin (IVIG) have been used to treat several viral infections. A substantial proportion of POTS is thought to be immune-mediated. A number of case reports show improvement with immune modulating therapy, particularly intravenous immunoglobulin (IVIG), in patients with various post-COVID conditions.[10,11]

Medications that are used to treat chronic heart failure with elevated HR are also potential treatment options for improving tachycardia symptoms in patients with PASC and POTS.

Apart from pharmacologic therapy, the current foundation of treatment for POTS, and for POTS in patients with PASC, is non-pharmacologic therapy, including volume expansion, behavioral and rehabilitation therapy. Specifically, based on small studies and pathophysiologic rationale, patients are treated with volume expansion with encouragement of fluid and salt intake, abdominal binders, and physical therapy directed at orthostatic symptoms. An important question is whether such therapy, if applied in a systematic and intense way with the assistance of a care coordinator, would be more effective than usual care in which these treatments may be recommended but without assurance of adherence. Thus, there is interest to assess the effect of coordinated non-pharmacologic, behavioral and rehabilitation care versus usual care.

Evidence in the current literature indicates that non-pharmacologic therapy in combination with immune therapies and cardiac modulators have promise in treatment of PASC-related autonomic symptoms. Randomized trial evidence is needed to determine if these treatments provide meaningful benefit. If successful, this trial program will rapidly provide the foundational evidence to enable providers to identify and treat patients with PASC and POTS to ameliorate these symptoms.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Potential risks of this study include those associated with the specific study intervention (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 6-minute walk test include tiredness, shortness of breath, or palpitations during the test. The active stand test and full autonomic testing may cause some participants to faint or feel lightheaded or weak.

The following risks are associated with the autonomic function tests:

- The head-up tilt (HUT) test may cause lightheadedness and fainting due to a drop-in blood pressure. However, there is no risk of falling from the table as the subject is restrained by heavy straps and the table will be lowered horizontally to relieve any dizziness.
- The Valsalva maneuver may lead to chest pain, hypotension, fainting, and abnormal heart rhythms.
- Deep breathing test may lead to shortness of breath and dizziness
- Skin biopsy is a minimally invasive procedure and is a generally safe procedure. Risks of obtaining a tissue biopsy are bleeding, bruising, scarring, soreness, redness, and infection at the tissue punch biopsy site. For the procedure, lidocaine with epinephrine is administered subcutaneously at the biopsy site. At this dose, there is minimal risk of systemic toxicity. Potential risks include allergic reactions and anaphylactic shock. The transcranial doppler is a form of non-invasive medical imaging. The risks associated with it are minimal and include discomfort or pressure from the probe.

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 improvement in PASC symptoms.

Society in general and future patients infected with SARS-CoV-2 may benefit from the study's results, which will provide a better understanding of the benefit/risk of non-pharmacologic and pharmacologic treatment options for autonomic dysfunction symptoms, including POTS, in PASC patients.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Potential benefits may outweigh potential risks. Autonomic dysfunction in 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 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).

While the primary outcome is OHQ/OIQ, the overarching goal is to determine if the interventions result in improved symptoms and quality of life. This will be evaluated by a number of questionnaires including COMPASS-31 and PROMIS-29, as well as tests of functional status such as a 6-minute walk test. As a phase 2 trial, the total evidence from the primary and secondary outcomes will provide the most complete assessment of the impact of the interventions.

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

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Primary		
Evaluate the effect of study intervention versus control on orthostatic intolerance	Orthostatic Hypotension Questionnaire (OHQ)/ Orthostatic Intolerance Questionnaire (OIQ)	Change from baseline to end of intervention (EOI)
Secondary		
Evaluate the effect of study intervention versus control on symptom-specific outcome measures	Composite Autonomic Symptoms Score 31 (COMPASS-31) Malmo POTS Symptom Score (MAPS)	Change from baseline to EOI
Describe the effect of study intervention versus control on performance-based and vital sign outcome measures	Heart rate (HR) measurements during Active Stand Test 6-min Walk Test	Change from baseline to EOI
Evaluate the effect of study intervention versus control on quality of life	PROMIS-29 + 2 Questionnaire	Change from baseline to EOI
Characterize the safety and tolerability of study intervention for treatment of PASC	Adverse events, including serious adverse events (SAEs) and events of special interest (ESIs)	Proportion of participants who experience individual SAEs and the proportion who experience any one or more SAEs Incidence of SAEs leading to discontinuation

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
		Incidence of ESIs
Exploratory		
Evaluate the effect of study intervention versus control on autonomic function tests	Autonomic function tests at specialized sites: • Head-up tilt (HUT) test • Valsalva maneuver with a pressure of 40 mmHg for 15 seconds • Deep breathing test • Skin biopsy (if applicable as per Appendix) • Transcranial doppler (TCD), if available	Change of quantitative autonomic symptoms score (QASAT) or sub-scores from baseline to EOI
Describe the effect of study intervention versus control on performance-based symptom measures	Vanderbilt Orthostatic Symptoms Score (VOSS)	Change from baseline to EOI
Describe the effect of study intervention versus control on PASC symptoms	PASC Symptom Questionnaire	Change from baseline to EOI

OBJECTIVES	OUTCOME MEASURES	ENDPOINTS
Evaluate the effect of study intervention versus control on relevant biomarkers	• Viral biomarkers • Autonomic function biomarkers • Immune biomarkers • Inflammatory biomarkers • Endothelial function biomarkers • Cognitive function (CCL11)	Change in biomarker(s) from baseline to EOI
Describe the effect of study intervention versus control on performance-based and vital sign outcome measures	Blood pressure (BP) measurements during Active Stand Test	Change from baseline to EOI
Compare the effect of study intervention versus control on specific tracked measurements	Wearable device measuring accelerometry (step count and HR)	Change from baseline to EOI

4 STUDY DESIGN

4.1 PLATFORM PROTOCOL DESIGN

This platform protocol is a prospective, multi-center, multi-arm, randomized, controlled trial evaluating treatment of autonomic dysfunction PASC symptoms in outpatients previously infected with SARS-CoV-2. This study is a platform protocol designed to be flexible so that it is suitable for a wide range of settings within healthcare systems and in community settings where it can be integrated into COVID-19 testing programs and subsequent treatment plans. The platform protocol will enroll participants meeting criteria for autonomic dysfunction symptoms. Each appendix will describe a study intervention that is sized to meet the platform protocol objectives. Participants will be randomized to pre-specified factorial combinations of a study drug and non-pharmacologic intervention along with their controls that are actively enrolling at the time of randomization. Interventions may be added or removed according to adaptive design and/or emerging evidence. Refer to the protocol Appendices for further information on each study intervention and study design.

Patient-reported outcomes (PROs) of symptoms and functional status and objective assessments will be done. Periodic clinic visits will be done during the intervention period and a follow-up assessment will be done 3 months after EOI.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

A randomized trial design is used to compare the intervention to control under a platform protocol. In addition, where applicable, double-blind will be maintained. A factorial design is used in each study intervention in combination with a non-pharmacologic care, where the non-pharmacologic care is not blinded due to its practical implementation.

Evidence in the current literature indicates that non-pharmacologic therapy and medications have promise in treatment of PASC-related cardiovascular symptoms. Randomized trial evidence is needed to determine if these treatments provide meaningful benefit.

Additional pharmacologic therapies can be added to this platform protocol as new drugs are available or as the understanding of PASC is improved and favorable drug properties are discovered.

Apart from pharmacologic therapy, the current foundation of treatment for POTS is non-pharmacologic therapy, including volume expansion, behavioral and rehabilitation therapy. An important question is whether such therapy, if applied in a systematic and intense way with the assistance of a care coordinator, would be more effective than usual care in which these treatments may be recommended but without assurance of adherence. Thus, this study design was selected to determine the effect of coordinated non-pharmacologic, behavioral and rehabilitation care versus usual care. It may be worthwhile to also explore the interaction effect of non-pharmacologic treatment concurrently with pharmacologic therapy.

4.3 JUSTIFICATION FOR DOSE

The duration (and dose if applicable) of the intervention will vary depending on the intervention. Refer to the appendices for details.

4.4 END OF STUDY DEFINITION

The End of Study (EOS) will occur when all participants have completed EOI and Follow-up 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:

- ≥ 18 years of age at the time of enrollment
- Previous suspected, probable, or confirmed SARS-CoV-2 infection, as defined by the Pan American Health Organization[12ϕ]

$^{\phi}$ Enrollment of participants with suspected or probable SARS-CoV-2 infection will only be allowed if they occurred before May 1, 2021, and be limited to no more than 20% of the total sample size per Study Drug Appendix. Refer to the Manual of Procedures (MOP) for details.
Suspected case of SARS-CoV-2 infection - Three options, A through C:

A. *Meets the clinical OR epidemiological criteria.*
    a. *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.*
    b. *Epidemiological criteria: Contact of a probable or confirmed case or linked to a COVID-19 cluster; or*
B. *Presents with 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. *Presents 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, defined as 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.*

- Moderate, self-identified autonomic symptoms (defined as COMPASS-31 >20) following a SARS-CoV-2 infection that has persisted for at least 12 weeks and is still present at the time of consent
- OHQ/OIQ, question 1 score >2

5.2 EXCLUSION CRITERIA

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

- Known pregnancy, breast-feeding, or contemplating pregnancy during the study period
- Known active acute SARS-CoV-2 infection ≤ 4 weeks from enrollment
- Known renal failure (eGFR <20ml/min/1.73 m²)
- Known cardiovascular conditions such as heart failure (Class 3-4) or severe valvular disease
- Clinically significant atherosclerotic disease, defined as history of stroke or myocardial infarction or revascularization 6 months prior to enrollment and/or current symptomatic angina
- Existing uncontrolled hypertension
- Inability to comply with the protocol

- Any condition that the investigator believes makes it inappropriate to treat with study intervention, including severe debility.
- History of POTS or significant dysautonomia before COVID-19 index infection.
- Enrolled in another clinical trial or another study intervention appendix in this platform protocol
  ** Patients who are a part of observational studies (e.g. imaging) may be included in this trial. Participants may enroll in another interventional trial or re-enroll in this trial for a different study intervention appendix 30 days after end of study (Follow-up visit).*

Exclusion criteria specific to each intervention are listed in the Appendices.

5.3 LIFESTYLE CONSIDERATIONS

Participants of child-bearing age must agree to use an effective method of contraception during study drug administration and for at least 7 days after their final dose of study drug. Effective methods include any of the following: abstinence, partner vasectomy, bilateral tubal ligation, intrauterine device, progestin implants, barrier (condom, diaphragm, cervical cap), oral contraceptive, or spermicide.

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 a 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. To support participant referral to actively enrolling trials, a series of invitation algorithms based on appendix-specific inclusion/exclusion criteria and participant entered data may be used. Automatic invitations will be generated for participants who appear eligible based on trial interest, demographics, and medical history. Once participants accept the invitation and adequate consent is obtained, their information will be shared with the applicable study team.

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 for pharmacologic interventions and Section 6.6 for non-pharmacologic intervention.

6.1.2 DOSING AND ADMINISTRATION

See appendices.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Receipt and use of any pharmacologic study intervention (i.e. study drug) will be handled, tracked by the sites, and stored in a safe and secure location to which only the investigator and designated personnel have access. Use of the study drug will be tracked by the sites. When the study intervention is a pill or tablet, the participants will be asked to bring the packaging to every clinic visit and sites will monitor adherence through pill/tablet count. Participants will also be asked to bring any unused study drug with them to the clinic during their EOI visit. Sites may also contact the participants at regular intervals during the study intervention period to monitor compliance.

See appendices for further details.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

See appendices.

6.2.3 PRODUCT STORAGE AND STABILITY

As applicable, upon receipt, study intervention 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

Since the route and duration of administration of each study intervention differs, the controls also differ. To achieve blinding and an equitable randomization probability, the following process will be used.

In each Appendix trial, each participant will be assigned with equal probability to one of the factorial combinations based on 2 factors: (1) a study intervention/control and (2) non-pharmacologic intervention/control if the participant is eligible for the study intervention. If more study drugs are added with the same administration route and the same inclusion and exclusion criteria with a shared control, the level of the study intervention factor may be m+1 level where m is the number of active study interventions along with one shared control. In that case, the study design will be an (m+1) x 2 factorial design.

Sites will be informed as to which appendix participants are randomized, but not whether they are allocated to the active study intervention arm or control arm within that appendix. The participants and investigators will be blinded throughout the study for the study intervention, but not for the non-pharmacologic intervention. Due to the nature of non-pharmacologic intervention, blinding is not possible.

6.3.1 UNBLINDING

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 is completed. Only the biostatistical team who is preparing closed interim reports will be unblinded. Specifically, pharmacologic intervention/control will be dispensed with packaging and labeling that would blind treatment assignment. Unblinding will occur only if required for participant safety or treatment, at the request of the treating clinician.

Refer to the MOP for further details.

6.4 STUDY INTERVENTION ADHERENCE

Participants will be notified of the importance of completing all aspects of their intervention.

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.

No medications or standard treatments for POTS will be withheld from study participants.

Refer to the Appendices for study-specific information on concomitant medications.

6.6 NON-PHARMACOLOGIC CARE

All participants will receive coordinated non-pharmacologic care or usual non-pharmacologic care (control) for a duration of 3 months. This will be concurrent to study drug intervention (refer to Appendices).

Coordinated non-pharmacologic care involves a systematic and intensified program of volume expansion through high salt diet (6-10 grams of salt per day), water intake (at least 2 liters [64 ounces] per day), abdominal binder, physical rehabilitation, weekly monitoring of BP, motivation, education, and assisted care through a care coordinator. The care-coordinators will be required to complete a training program, and details will be provided in the MOP.

All participants receiving coordinated non-pharmacologic care will receive a kit of equipment needed. Additional details are in the MOP.

The care coordinator will call participants in coordinated non-pharmacologic care arm weekly during the 3-month non-pharmacologic intervention period to provide education and support on lifestyle modifications. Participants will be asked to maintain a weekly log of non-pharmacological care (average dietary intake of salt and water, physical rehabilitation) and provide a summary to the care-coordinators during the weekly calls.

To abort lightheadedness episodes, participants will be encouraged to sleep with their head elevated (6-12 inches of elevation) and drink 16 ounces of water (about ½ liter) in the morning while in bed before standing. In addition, practicing physical countermeasures (such as leg crossing, buttock clenching, squatting, squeezing a stress ball repeatedly, or clasping and pulling the fists apart) will be encouraged with the goal of further helping to prevent lightheadedness. Participants will also be informed to avoid triggering events such as prolonged standing in one position, hot environments (hot showers), sudden standing after waking in the morning, prolonged fasting, excessive alcohol intake and straining (during urination or bowel movements).

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 study protocol. If a clinically significant finding is identified (such as changes from baseline) after enrollment, 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 study drug would not be in the best interest of the participant
- Confirmed new case of acute SARS-CoV-2

Participants will be followed for safety even if study intervention is discontinued. The reason for participant discontinuation from study intervention will be recorded on the CRF. Participants who are discontinued from the study intervention, but who are not withdrawn from the study will continue to be followed for all study procedures.

Participants who discontinue the study drug, but do not withdraw consent, will be followed for safety for at least 28 days after the final study drug administration. Participants will continue to receive non-pharmacologic care even if study drug intervention is discontinued. Participants may be replaced with additional randomized participants but will not be specifically matched with the withdrawn participant.

7.2 PARTICIPANT WITHDRAWAL FROM THE STUDY

Participants are free to withdraw consent 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 consent 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 study file.
- Should the participant continue to be unreachable, after exhausting all methods, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7.4 HALTING AND STOPPING RULES

The sponsor has engaged Duke Clinical Research Institute (DCRI) Safety Surveillance to oversee real-time serious adverse event (SAE) collection, evaluation, and expedited regulatory reporting for this study. An independent DCRI Safety Medical Monitor will be responsible for evaluating causality assessment and expectedness of site reported SAEs compared to the product label. Details of this process can be found in the study-specific Safety Management Plan.

Participant safety will be reviewed by RECOVER Data and Safety Monitoring Board (DSMB) that is composed of individuals with the appropriate expertise. Members of the DSMB will be independent from the study conduct and free of conflict of interest, and measures will be in place to minimize perceived conflict of interest. The DSMB will meet as stated in their charter 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 sponsor.

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

<u>A participant's study drug will be permanently discontinued if:</u>

- The participant requests to stop study drug
- The investigator believes that, for safety reasons or tolerability reasons, it is in the best interest of the participant to discontinue study drug
- The participant has a serious (i.e. SAE) or severe (Grade 3 or above) anaphylactic or allergic reaction that is determined to be related to the study medication

Permanent discontinuation of study drug should be considered for patients with side effects possibly related to study drug such as thrombosis, worsening renal function, aseptic meningitis, hemolytic anemia, and transfusion related lung injury.

The overall trial would be paused, pending DSMB review for:

- Two confirmed anaphylactic reactions determined to be related to study drug
- Two episodes of thrombosis, including deep venous thrombosis and/or embolic events, determined to be related to study drug
- Two episodes of renal failure that is not otherwise explained by the subject's co-morbidities
- Two episodes of aseptic meningitis syndrome
- Two episodes of hemolytic anemia requiring transfusion
- Two episodes of transfusion related acute lung injury determined to be related to study drug
- Two deaths determined to be related to study drug
- Two of any other SUSARS

The overall trial will be stopped if:

- Sponsor determines that the study should be stopped based upon recommendations from DSMB and NIH (e.g. based on unblinded assessment of risk and benefit, including imbalance of serious or severe allergic reactions, anaphylaxis, deaths, and SUSARs).

Circumstances that may warrant termination or suspension may also include protocol compliance and data quality issues:

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Please refer to the Appendices for any further specific halting and stopping rules.

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

No formal stopping rules for efficacy will be implemented in this study because it is unlikely that there will be such a large benefit detected given the modest sample size and short duration. As above, study intervention may be suspended for serious safety concerns, after considering the balance of efficacy and safety. The DSMB recommendations will be considered by the sponsor, NIH, and the study Principal Investigator prior to making any decisions regarding study continuation or discontinuation.

No ESIs are recorded as part of the master protocol, but ESIs will be documented in each study appendix depending upon the study intervention (Refer to Appendices).

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCHEDULE OF PROCEDURES

Table 2. Schedule of Study Procedures: Platform Protocol

	Screening[1]	Baseline/ Randomization [2,3]	Study Intervention Period[4]	End of Intervention (EOI) Clinic Visit	Early Termination Clinic Visit	Follow-up Visit[5]
PROCEDURE	**Day -28 to -7**	**Day -10 to -7**	**Day 1 to EOI**	**Refer to Appendix**		**EOI + 3 months (± 2 weeks)**
Platform Protocol Eligibility Criteria (Overall)	X		Refer to Appendix-specific study procedures	Refer to Appendix-specific study procedures		
Informed consent	X					
Demographics	X					
Medical History[6]	X					
Concomitant Medications	X	X			**X**	
Randomization		X				
Coordinated Non-pharmacologic Care[7]						
Appendix Eligibility Criteria	X	X				
Nasal Swab for SARS-CoV-2 rapid antigen test		X				
Clinic visits, as per specific study Appendix					X	X
OHQ/OIQ	X	X[8]			X	X
COMPASS-31	X				X	X
Malmo POTS Symptom Score (MAPS)		X			X	X

	Screening[1]	Baseline/ Randomization [2,3]	Study Intervention Period[4]	End of Intervention (EOI) Clinic Visit	Early Termination Clinic Visit	Follow-up Visit[5]
PROCEDURE	**Day -28 to -7**	**Day -10 to -7**	**Day 1 to EOI**	**Refer to Appendix**		**EOI + 3 months (± 2 weeks)**
Vanderbilt Orthostatic Symptoms Score		X		Refer to Appendix-specific study procedures	X	X
Active Stand Test		X	Refer to Appendix-specific study procedures		X	X
6-minute Walk Test		X			X	X
Modified DePaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM)[9] (look back period of 7 days)		X				
DSQ-PEM[9] (look back period "since last visit")		X			X	X
PROMIS-29 + 2 Questionnaire		X			X	X
PASC Symptom Questionnaire		X			X	X
Blood Collection[10]		X			X	X
Stool Collection		X			X	
Skin Biopsy[11]		X			X	
Autonomic function tests at specialized sites		X			X	
Pregnancy Test		X				
Height and weight		X				

	Screening[1]	Baseline/ Randomization [2,3]	Study Intervention Period[4]	End of Intervention (EOI) Clinic Visit	Early Termination Clinic Visit	Follow-up Visit[5]
PROCEDURE	**Day -28 to -7**	**Day -10 to -7**	**Day 1 to EOI**	**Refer to Appendix**		**EOI + 3 months (± 2 weeks)**
Study Drug Administration						
Distribution of wearable[12]		X				
Safety Assessment of AEs including SAEs and ESIs		X			X	X
Adherence[13]					X	

Abbreviations: PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ-Orthostatic intolerance questionnaire; AE-Adverse Events; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1]Some of the eligibility baseline assessments (active stand test and labs), can be performed at the Screening visit if the participant comes in-person for Screening.

[2] Screening cannot be on the same day as the Baseline/Randomization visit.

[3] Eligibility assessments (active stand test, OHQ/OIQ, and labs) should be completed prior to randomization.

[4]A detailed schedule of activities for the Study Intervention Period is provided in each Appendix.

[5] Follow-up visit windows for each appendix:

Appendix A: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 336 (Day 322 to Day 350)

Appendix B: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 168 (Day 154 to Day 182)

[6] If medical Records are not available, they are not required. History and physical examination could be performed at screening/baseline visit. At every clinic visit after screening visit, changes in medical history including SARS-CoV-2 infection since last visit will be collected.

[7]Only applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only. During this time, a care coordinator will reach out to the participants weekly.

[8] The OHQ/OIQ completed at Baseline visit will be used to determine eligibility.

[9] The DSQ-PEM will be done twice for the baseline visit: once on the day of the visit with a look back period of “past 7 days” and once within one day after the visit with a look back period of “since last study visit (SLV)”. DSQ-PEM SLV will be administered via phone or electronic survey. The DSQ-PEM administered at every timepoint after baseline will be within one day after strenuous in-person study visits and will ask about the period “SLV”.

[10] Blood collection for safety parameters, biomarkers, and biospecimen for biorepository. Blood collection may also be collected as needed based on PI discretion and adverse events.

[11] Skin biopsy is only applicable to Appendix A: IVIG. If the baseline skin biopsy shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET.

[12] It is required to wear the wearable at least 7 days before start of study drug intervention and 7 days before end of intervention visit. All other times, it is strongly encouraged.

[13] Adherence to drug (pill count) will be documented. Participants receiving oral study drugs to be taken at home will receive weekly phone calls for the first 30 days.

8.2 SCREENING (DAY -28 TO -7)

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.

It is required to have completed the Screening prior to the Baseline/Randomization visit. Screening cannot be on the same day as the Baseline/Randomization visit. After the informed consent, the following assessments will be performed at screening to determine eligibility:

- Demographics
- Medical History
    - Including SARS-CoV-2 test result date (if available), signs and symptoms, and treatment (including hospitalization, Intensive Care Unit (ICU) status, supplemental O_2 status), COVID-19 vaccination status and dates, and PASC history (symptoms and duration).
    - At every clinic visit after screening visit, changes in medical history including SARS-CoV-2 infection since last visit will be collected.
    - If medical records are not available, they are not required. History and physical examination could be performed at screening/baseline visit.
- Collection of concomitant medications taken within 14 days of informed consent
- COMPASS-31
- OHQ/OIQ
- Review appendix-level eligibility criteria, refer to Appendices

8.3 BASELINE/RANDOMIZATION (DAY -10 TO -7)

The following will occur at the Baseline/Randomization visit:

- Concomitant Medication review
- OHQ/OIQ
- MAPS
- VOSS
- Active Stand Test
- 6-minute walk test
- Modified DePaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM)
    - At Baseline, this will be done twice: once on the day of the Baseline visit with a look back period of “past 7 days” and once the day after the Baseline study visit with a look back period of “since last study visit (SLV)”. DSQ-PEM SLV will be administered via phone or electronic survey.
- Autonomic function tests, at specialized sites

- Skin biopsy will only be done in Appendix A: IVIG. If the baseline result shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET.
- PROMIS-29 + 2 Questionnaire
- PASC Symptom Questionnaire
- Nasal Swab for SARS-CoV-2 rapid antigen test
- Blood collection for the following evaluations:
    - Complete Blood Count (local, if not done within the past 3 months)
    - Comprehensive Metabolic Panel (local, if not done within the past 3 months)
    - Biomarkers that may include viral biomarkers, immune biomarkers, Inflammatory biomarkers, Endothelial function biomarkers, and biomarkers for autonomic and cognitive function). Refer to Section 8.9.12.
- Biospecimens (serum, plasma, and stool) to be stored in biorepository (frozen for retrospective analysis). Refer to Section 8.10. Pregnancy test, urine in individuals of child-bearing potential
- Height and weight
- Distribution of wearable, it is required to wear the wearable 7 days before start of study drug intervention and 7 days before end of intervention visit. All other times, it is strongly encouraged.
- Randomization: 1:1:1:1 randomization to combination of study drug intervention/control with coordinated non-pharmacologic care/usual care
- Safety assessment of SAEs
- Review appendix-level eligibility criteria, refer to Appendices

8.4 STUDY INTERVENTION PERIOD (DAY 1 TO END OF INTERVENTION [EOI])

Refer to Appendices for study drug-specific intervention duration. The following will occur during the drug dosing period:

- Concomitant Medication review
- Study intervention administration, as per specific study Appendix
- Coordinated non-pharmacologic care or usual care, as applicable. Refer to Section 6.6.
- Clinic visits, as per specific study Appendix
- OHQ/OIQ
- COMPASS-31
- MAPS
- VOSS
- Active Stand Test
- 6-minute Walk Test
- DSQ-PEM
- PROMIS-29 + 2 Questionnaire

- PASC Symptom Questionnaire
- Safety assessment of AEs, including non-serious AEs, SAEs and ESIs
- Height and weight, as applicable for dosing
- Vital signs monitoring
- Adherence to study intervention(s)
    - Care-coordinators will reach out weekly to the participants randomized to coordinated non-pharmacologic care
    - Pill count and weekly phone calls (for the first 30 days) for participants receiving oral study drugs to be taken at home

8.5 EARLY TERMINATION CLINIC VISIT

- Concomitant medication review
- OHQ/OIQ
- COMPASS-31
- MAPS
- Active Stand Test
- VOSS
- 6-minute Walk Test
- DSQ-PEM
- PROMIS-29 + 2 Questionnaire
- PASC Symptom Questionnaire
- Blood collection for safety parameters (complete blood count [CBC] with differential and complete metabolic panel [CMP]), biomarkers, and biospecimens for biorepository, including distribution of stool sample kit
- Skin biopsy, if applicable
- Autonomic function tests at specialized sites
- Safety assessment of AEs, including non-serious AEs, SAEs and ESIs
- Adherence to study interventions

8.6 END OF INTERVENTION CLINIC VISIT (DAY EOI + 1 WEEK)

The EOI Clinic Visit will occur after the final study intervention administration. The following will occur at the EOI Clinic Visit:

- Concomitant Medication review
- OHQ/OIQ
- COMPASS-31
- MAPS
- VOSS
- Active Stand Test
- 6-minute Walk Test
- DSQ-PEM
- PROMIS-29 + 2 Questionnaire
- PASC Symptom Questionnaire
- Blood collection for safety parameters (CBC with differential and CMP), biomarkers, and biospecimens for biorepository, including distribution of stool sample kit
- Autonomic function tests at specialized sites
- Skin biopsy, if applicable
- Safety assessment of AEs, including non-serious AEs, SAEs and ESIs
- Adherence to study interventions

8.7 FOLLOW-UP (EOI + 3 MONTHS)

Follow-up clinic visits will occur 3 months after EOI. The following assessments will occur as part of follow-up:

- OHQ/OIQ
- COMPASS-31
- MAPS
- VOSS
- Active Stand Test
- 6-Minute Walk Test
- DSQ-PEM
- PROMIS-29 + 2 Questionnaire
- PASC Symptom Questionnaire
- Blood Collection for biomarkers and biospecimens for biorepository
- Safety assessment of SAEs and ESIs

8.8 CLINICAL LABORATORY ASSESSMENTS

A CBC with differential and CMP are required as part of baseline study 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.

Blood will be collected from participants and sent to a central lab for biomarkers assessment (refer to Section 8.9.12)

8.9 STUDY ASSESSMENTS

8.9.1 ORTHOSTATIC HYPOTENSION QUESTIONNAIRE

The Orthostatic Hypotension Questionnaire (OHQ) is a PRO that measures orthostatic intolerance, which has been the primary presentation of patients with PASC-related autonomic dysfunction. This measure includes the 6-item symptom assessment (OHSA) and the 4-item Daily Activity Scale (OHDAS). Each item is scored from 0 (none/no interference) to 10 (worst possible/complete interference), describing the preceding week. Prior data in patients with PASC suggests that this measure well-discriminates those with symptoms compared to healthy controls. Measurements for the first 50 subjects will be used for test-retest reliability.

8.9.2 COMPASS-31

The COMPASS-31 is a PRO that measures autonomic symptoms across multiple domains commonly seen in patients with PASC.

8.9.3 MALMO POTS SYMPTOM SCORE (MAPS)

The MAPS assesses symptom burden in POTS. It is a self-rating, 12-item score (0-10 per item, total range 0-120) based on patients' own perception of symptoms through visual analogue scale assessment.[13]

8.9.4 ACTIVE STAND TEST

The active stand test is a well-characterized performance measure of short-term neurologic and cardiovascular function that requires limited resources and aids in diagnosis of patient symptoms of orthostatic intolerance.

It is important to standardize and measure change from supine to standing and not from sitting with legs down to standing. Participants will remain supine for 10 minutes, and data will be acquired at 5 and 10 minutes. Standing test should be performed with HR and BP monitoring at 1, 3, 5 and 10 minutes. In patients with orthostatic intolerance this test induces a BP drop within the first minute that correlates with HR and BP responses to tilt up and can be used as a measure of orthostatic intolerance.[14]

8.9.5 VANDERBILT ORTHOSTATIC SYMPTOMS SCORE (VOSS)

The VOSS consists of 9 orthostatic symptoms rated on a scale of 0 (no symptom) to 10 (worst the participant has experienced) at the end of each HUT and after the active stand test. These symptoms are mental clouding, blurred vision, shortness of breath, rapid heartbeat, tremulousness, chest discomfort, headache, lightheadedness, and nausea.

8.9.6 6-MINUTE WALK TEST

Normal walking speed will be measured using a standard 6-minute walk, which is an established measure of overall health.[15]

8.9.7 MODIFIED DEPAUL SYMPTOM QUESTIONNAIRE-POST EXERTIONAL MALAISE

The Modified DePaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM) PRO assessment.[16] Some patients have experienced debilitating PEM that may occur following exertion or on the day after exertion. To assess PEM, the DePaul Symptom Questionnaire 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 7-day look back period at baseline, and at all other timepoints, the look back period will be since the last study visit.

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 if the participant reports having at least one moderate (rated severity ≥ 2) PEM symptom for a frequency rated ≥ 2.

The DSQ-PEM questionnaire will be via phone or electronic survey within one day after strenuous in-person study visits. This evaluation will allow us to assess the effect of study procedures on exacerbation or development of PEM in a population who may be particularly vulnerable to this condition. For the purpose 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.

8.9.8 PROMIS-29+2

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.[17] 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. PROMIS-29+2 is the PROMIS-29 Profile v2.1 and two items from Cognitive Function – Abilities v2.0. The PROMIS global

health scales has been correlated against the EuroQol EQ-5D.[18] Additionally, PROMIS scales have been used with PASC patients.[19]

8.9.9 SKIN BIOPSY

This will be required for all participants enrolled in specific Appendices only. This test evaluates intraepidermal small fiber density and sudomotor fiber density in distal, intermediate, and proximal legs. If the baseline result shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET.

8.9.10 AUTONOMIC FUNCTION TESTS AT SPECIALIZED SITES

Additional autonomic function testing will be completed at specific sites that have the capability to perform these tests:

- Head-up Tilt (HUT) Test - This test stimulates the autonomic nervous system with orthostatic stress by passive movement from a supine position to an upright tilt in a controlled laboratory setting. Tilt test provides both sympathetic and parasympathetic assessments. The variables measured will be continuous BP, HR, end tidal CO_2, and cerebral blood flow velocity (CBFv) using transcranial doppler monitoring (CBFv and end tidal CO2 only at sites that have the appropriate device). The technique used for this assessment will be 10 minutes of baseline and 10 minutes 70-degree head-up tilt (HUT). The outcome will be all variables at baseline and at every 1 minute of the tilt (total 10 measurements during the tilt).
    - Transcranial doppler (TCD), if available – The test measures the velocity of blood flow through the brain's blood vessels by measuring the echoes of ultrasound waves.
- Valsalva maneuver – This test evaluates sympathetic adrenergic functions using BP responses and cardiovagal (parasympathetic) functions using the HR responses. Valsalva maneuver consists of forced expiration against the resistance with the expiratory pressure during strain at 40 mm Hg for 15 seconds. The measured variables will be continuous BP and HR.
- Deep breathing test – The test evaluates changes in the instant HR that is provoked by “deep” breathing. The test is performed in a supine position. The measured outcomes will be the ratio of the HR during inspiration and expiration as well as the calculated respiratory sinus arrhythmia (RSA). RSA amplitude is defined as the difference between the end of expiration and end of inspiration in HR. Typically, 6 respiratory cycles are identified, and the respective RSA amplitudes are averaged.

The final outcome of these measures is the quantitative autonomic symptoms score (QASAT). The total and sub scores (cardiovagal, adrenergic, respiratory, cerebral blood flow, small fibers, and autonomic failure) will be obtained.

8.9.11 PASC SYMPTOM QUESTIONNAIRE

Participants will be asked to complete a questionnaire that asks about the presence of PASC symptoms. This questionnaire includes additional PASC symptoms that are not directly related to autonomic dysfunction. Two questions, patient global impression of severity (PGI-S) and change (PGI-C) are included in this questionnaire.

8.9.12 BIOMARKERS

Blood will be collected from participants and sent to a central lab for biomarkers assessment. The biomarkers assessed will include inflammatory cytokines, skin biopsies, and other viral biomarkers, immune biomarkers, Endothelial function biomarkers, and biomarkers for autonomic and cognitive function.

Refer to the MOP for further details regarding specimen collection, preparation, and shipment.

8.10 BIOREPOSITORY FOR FUTURE RESEARCH

The RECOVER Biorepository is designed to collect and store blood plasma, serum samples, and stool samples for future research related to the various studies of the RECOVER Program. Such research might include developing diagnostic and/or prognostic tests, improving our understanding of the underlying pathophysiology of PASC, and developing new therapeutic targets. 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 DCRI which serves as the CT - DCC for all RECOVER clinical trials.

The existence of the biorepository will provide the opportunity to devise new hypotheses, since blood collection techniques are standardized across all approved protocols thereby allowing cross-protocol sample comparisons if scientifically justified.

Within this framework, the design of the biorepository is to collect additional blood and prepare EDTA plasma and serum, to be stored in the biorepository for future, as yet unspecified, analyses and studies. A serologic test of COVID-19 infection will be done as part of the biorepository for all participants. Approximately eighty (80) mL of blood will be collected to prepare the aliquots for storage at -80°C. Specifically, two 1.0 ml aliquots of EDTA plasma and two 1.0 ml aliquots of serum will be prepared. These samples will be stored at the Biorepository in a lab for up to 7 years.

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 pre-existing condition that did not worsen from baseline does not meet the definition of an SAE.

An unanticipated problem involving risk to non-participants, including environmental exposure and exposure to a breast-feeding infant, will be reported to the sponsor and the site IRB and central IRB, as appropriate. An event occurring in a non-participant will not be entered into the electronic data capture (EDC) system. Refer to the MOP 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 CRF (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) will be collected from the first invasive study procedure (blood collection or nasal swab) through the end of the Follow-up period (EOI = 3 months). 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

Each study drug appendix will identify whether all non-serious AEs will be collected to the study database from Start of study drug administration through the end of study drug dosing. Any non-serious AEs that result in study drug discontinuation will be identified as the reason for study drug discontinuation in the study database and reported as an AE. Any non-serious AEs that are ESIs will be collected from the start of study drug administration through the end of follow-up period.

9.1.2.1 SEVERITY OF EVENT

For reportable events, the following guidelines will be used to describe severity grades, described in the Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published November 27, 2017.

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

9.1.2.2 RELATIONSHIP TO STUDY INTERVENTION

All reportable events must have their relationship to the study intervention assessed by the by site investigator 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 an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Adverse events (AEs) 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 or DSMB 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 US 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, if applicable 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 suspected unexpected serious adverse reaction (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 notes 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. On a monthly basis, the DSMB chair will receive a report of the listing and overall pattern of SAEs, including hospitalizations. Refer to the Safety Management Plan for further details regarding specific reporting timelines. In addition, see other sections (7.4 and 13.10) for guidelines for pausing or stopping drug in individual patients and the guidelines for pausing the trial while the DSMB reviews the safety data to make their recommendation.

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.

Events of special interest (ESIs) meeting serious criteria will be reported to the DSMB chair within 1-2 business days, and all events (non-serious AEs/SAEs) will be reported to the DSMB chair via a monthly listing.

9.1.6 REPORTING OF PREGNANCY

Pregnancies that occur following the first study drug administration while on-study will be collected. If the study drug appendix excludes pregnancy, 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 pharmaceutical partners if applicable of a pregnancy within 1-2 business days of receipt that occur involving the specific appendix of the supplied study drug/control, as required.

10 STATISTICAL ANALYSES

All statistical analyses will be performed using SAS (SAS Institute, Inc. Cary, NC, USA) version 9.4 or higher. Baseline demographic and clinical data will be summarized by the intervention arm. Descriptive statistics will include mean, standard deviation, median, 25th and 75th percentiles for continuous variables and frequency and percentage for categorical variables. Statistical comparisons will be

performed using two-sided tests at 0.05 significance level. Additional details regarding statistical analyses will be provided in the SAP that is available prior to first patient enrollment and that will be finalized prior to the database lock.

10.1 STATISTICAL HYPOTHESES

Primary Endpoint:

1. Improvement in OHQ/OIQ composite score at the EOI compared to baseline in intervention(s) vs. control.

Secondary Endpoints:

1. Improvement in symptom-specific outcomes (COMPASS-31 and MAPS) at the EOI compared to baseline in intervention(s) vs. control
2. Improvement in performance-based and vital sign outcome measures (Active Stand Test, 6-minute Walk Test) at the EOI compared to baseline in intervention(s) vs. control
3. Improvement in quality of life (PROMIS-29+2 summary score) at the EOI compared to baseline in intervention(s) vs. control
4. Study drug(s) are safe in the PASC population

10.2 SAMPLE SIZE DETERMINATION

This study uses an adaptive platform trial design that will allow study drugs to be added or dropped from consideration based on accruing evidence. Initial sample size estimates are based on 2 trials (Appendix A and Appendix B), each with a 2 x 2 factorial design, for each study drug in combination with non-pharmacologic intervention in a 2 x 2 factorial design. With 4 different combinations in the trial, this results in 1:1:1:1 random allocation. If additional study drugs with the same route are added later in the study drug factor in any of the 2 x 2 factorial studies that can utilize a shared control, the design may be (m+1) x 2 factorial design with m active study drugs and the sample size will be adjusted accordingly.

The primary interest of studies in this platform protocol is to evaluate the effect of the study interventions vs control. Studies in this platform design will also explore the potential interaction of study drug and non-pharmacologic intervention. However, we do not expect to have interactions among effects of the study drug with effects of non-pharmacologic care. The primary comparisons are the main effects of the interventions, i.e., study drug vs. placebo and coordinated non-pharmacologic care vs. control (usual care) within each study appendix. Power calculation is provided for each study appendix based on sample size without adjusting for multiplicity because the study is phase II where we try to identify potential efficacy. Furthermore, the main effect comparisons are considered in a factorial design where the degree of need for multiplicity adjustment is low due to the comparisons are nearly statistically independent due to orthogonal contrasts of the cells if the variances in the different cells are equal.[20]

For the primary comparisons in each appendix, the power is calculated to detect a clinically significant difference of one unit (equivalent to effect size of 0.5 based on SD of 2) in changes of OHQ/OIQ composite score between any of the 2 groups, assuming a missing date rate with 0.05 type I error with a

2-sided test. Further details on sample size, power and missing data rate are found in the specific appendices.

Our justifications for the above assumptions are based on literature. The clinically significant difference is chosen to be one unit in OHQ/OIQ composite score because the estimated minimally important difference (MID) ranges from 0.8 to 1.0 in a validation study of OHQ/OIQ.[21] The assumption of standard deviation (SD) of 2 in change of OHQ/OIQ composite score is based on the observed SDs of 2.07 (active arm) and 1.69 (control arm) in change of OHQ/OIQ composite score within 7 days in a phase 3 randomized, placebo-controlled clinical trial on droxidopa for neurogenic orthostatic hypotension.[22] The observed SD of OHQ/OIQ composite score in 48 POTS subjects is 1.68 and we would have SD of 2 or 2.1 in change of OHQ/OIQ composite score if the correlation between OHQ/OIQ composite score at baseline and the EOI is 0.3 or 0.2.

10.3 POPULATIONS FOR ANALYSES

Population for the primary efficacy analysis will be based on an intention-to-treat (ITT) population, consisting of all randomized participants. All randomized participants will be included and will be analyzed according to their randomly assigned treatment group. Population for safety analyses will consist of all randomized participants who receive at least part of the randomized study intervention or control.

10.4 STATISTICAL ANALYSES

This section describes analysis methods for the primary, secondary, safety and exploratory outcomes. Full details will be provided in the SAP. If additional study drugs are added later, the statistical analysis section will be revised appropriately.

10.4.1 ANALYSIS OF THE PRIMARY ENDPOINT

For primary comparison, the analysis population includes all randomized participants to study drug intervention or its control, and the primary endpoint is the change of OHQ/OIQ composite score from baseline to the EOI.

The same analysis approach will be used in all these primary comparisons. A regression-based approach will be used for the primary analyses where the change of OHQ/OIQ composite score is the dependent variable. A group indicator for coordinated care, a group indicator for IVIG , the baseline OHQ/OIQ composite score, and other pre-specified baseline variables will be the covariates (independent variables). Details will be provided in the SAP.
In addition to these primary analyses, we will also perform 2 exploratory analyses as a supplement. Specifically, we will include the testing of interaction between pharmacologic and non-pharmacologic treatments within each trial. Furthermore, we will assess the equality of non-pharmacologic treatment effect by testing the interaction of non-pharmacologic treatment and the trial indicator. Details will be provided in the SAP.

10.4.2 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Symptom-specific outcomes (COMPASS-31 and MAPS). Each of the symptom-specific outcomes is a continuous measure. Changes from baseline to EOI for each outcome will be analyzed using similar methods as those outlined for the primary endpoint. Our pilot preliminary data showed that COMPASS-31 has mean (SD) of 33.41 (18.92) in subjects with long COVID-19 and POTS. A 30% reduction in COMPASS-31 is considered to be clinically significant. This implies that the 10-unit difference is clinically significant if the mean COMPASS-31 is 34 at the baseline. The power for this secondary outcome is provided in the appendix.

Performance-based and vital sign outcome measures (HR during Active Stand Test, 6-minute Walk Test). Each of the performance and vital sign -specific outcomes is a continuous measure. Changes from baseline to EOI for each outcome will be analyzed using similar methods as those outlined for the primary endpoint.

Quality of life (PROMIS-29+2). Each of the symptom-specific outcomes is a continuous measure. Changes from baseline to EOI for each outcome will be analyzed using similar methods as those outlined for the primary endpoint.

Safety endpoint. Safety endpoints include the proportion of participants who experience individual SAEs and the proportion who experience any one or more SAEs. These will be analyzed in the safety population. Events of Special Interest (ESIs) will be summarized by study drug appendix and duration. Incidence of AEs/SAEs leading to discontinuation will also be summarized.

10.4.3 ANALYSIS OF THE EXPLORATORY ENDPOINT(S)

The quantitative autonomic symptoms score (QASAT) from the autonomic function tests, VOSS score after the active test or HUT, BP during Active Stand Test, PASC symptom questionnaire scores; biomarkers (viral, autonomic function, immune, inflammatory and endothelial function biomarkers), specific tracked accelerometry measurements (step count, HR), and cognitive function (CCL11) are the exploratory endpoints. Each of the outcome is a continuous measure. Changes from baseline to EOI for each outcome will be analyzed using similar methods as those outlined for the primary endpoint.

10.4.4 MISSING DATA

For the primary analysis, missing outcome data will be handled in accordance with prespecified methods outlined in the SAP. These methods will be chosen to support valid inference and may include model-based or other principled approaches that use available data to mitigate the impact of missingness.

Additional sensitivity analyses will be performed to assess the robustness of results and will be described in the SAP. The SAP will be finalized prior to database lock and unblinding of treatment assignments and will specify the primary and sensitivity approaches to missing data handling.

10.4.5 PLANNED INTERIM ANALYSES

Interim efficacy or futility analyses are not planned. Interim examination of clinical endpoints and key safety events will be performed at regular intervals during the course of the trial. An independent, NIH-appointed, DSMB will monitor participant safety and review 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.

This protocol does not have planned early stopping rules for efficacy nor for futility. 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. Stopping early would also limit the collection of data that are critical for planning future trials in similar patient populations.

10.4.6 PLANNED TEST-RETEST RELIABILITY ANALYSES

Subjects will be asked to fill out the OHQ/OIQ questionnaire twice, once at screening and once at baseline. The data from the first 50 subjects with 'No Change' status in the PGI-C will be used for the test-retest reliability analyses. Details will be provided in the SAP.

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 drug/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 drug and the desire to withdraw consent for study follow-up.

11.1.2 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.

11.1.3 KEY ROLES AND STUDY GOVERNANCE

The RECOVER program is overseen by the RECOVER Executive Committee. 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.

11.1.4 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.

11.1.5 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.

11.1.6 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.

11.1.7 DATA HANDLING AND RECORD KEEPING

11.1.7.1 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.

11.1.7.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 6 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.

11.1.8 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.

11.1.9 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.

11.1.10 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
BP	Blood Pressure
bpm	Beats per minute
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus Disease of 2019
CRF	Case Report Form
CT-DCC	Clinical Trial – Data Coordinating Center
DCRI	Duke Clinical Research Institute
DSMB	Data Safety Monitoring Board
EOI	End of Intervention
ESI	Events of Special Interest
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors

ICU	Intensive Care Unit
IND	Investigational New Drug Application
IRB	Institutional Review Board
MAPS	Malmo POTS Symptom Score
mITT	Modified Intention-To-Treat
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
OHQ	Orthostatic Hypotension Questionnaire
OIQ	Orthostatic Intolerance Questionnaire
PASC	Post-acute Sequelae of SARS-CoV-2 Infection
PHI	Personal Health Information
PRO	Patient-Reported Outcome
QASAT	Quantitative Autonomic Symptoms Score
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
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
US	United States
VOSS	Vanderbilt Orthostatic Symptoms Score

11.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change
1.0	22SEPT2023	None, original protocol
2.0	06OCT2023	• IND numbers were added for both appendices in the protocol title page • Updated Table 1 by removing “ambulatory” specification from BP and HR measurements • Moved VOSS and the PASC Symptom Questionnaire from secondary to exploratory outcomes; all relevant sections were updated accordingly • Consistent with other RECOVER protocols, updated exclusion criteria #14 in Section 5.2 with the following text and note: *Enrolled in another clinical trial or another study intervention appendix in this platform protocol*

		**Participants may enroll in the trial or re-enroll in the trial for a different study intervention appendix if they have completed a 30-day washout period. For those who wish to re-enroll, re-enrollment can only occur after efficacy has been determined for the appendix in which they were previously enrolled.* • Non-pharmacologic care details that are no longer relevant were removed from Section 6.6. Reference to the MOP was added for specific details. • Table 8, Footnote # 7 regarding skin biopsy was updated for further clarity: *Skin biopsy is only applicable to Appendix A: IVIG. If the baseline skin biopsy shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET* • The description for DSQ-PEM was updated throughout the protocol to remove the phrase "*remote follow-up*" • The description of QHQ/OIQ was updated to reflect the appropriate look back periods: 7 day look back period at baseline and at all other timepoints, the look back period will be since last visit • Section 9.1.5 was updated with the following statement: *Events of special interest (ESIs) meeting serious criteria will be reported to the DSMB chair within 1-2 business days and all events (non-serious AEs/SAEs) will be reported to the DSMB chair via a monthly listing.* • Following statement was added to Section 8.2 to describe the Screening and Baseline visit: *It is required to have completed the Screening prior to the Baseline/Randomization visit. Screening cannot be on the same day as the Baseline/Randomization visit.* • Updated description of active stand test: *Participants will remain supine for 10 minutes, and data will be acquired at 5 and 10 minutes. Standing test should be performed with HR and BP monitoring at 1, 3, 5 and 10 minutes.* • Updated QASAT score description for autonomic function testing. • Added clarity to the section on VOSS to indicate that it will be done after at the end of each HUT and after the active stand test.

		• Added indication for blood collection to the End of Intervention study visit in the Schedule of Procedures tables in Appendix A and B, along with the following footnote: *Blood may be collected at any time as needed based on PI discretion and adverse events.* • Modified the wording of the dosing information for IVIG in Appendix A as follows: *The dosing frequency of 0.5 g/kg once a week is strongly suggested; however, the frequency can be modified based on individual site preference* • Updated description of placebo capsules in Appendix B for consistency with manufacturing details • Added additional exclusion criteria to Appendix B based on FDA recommendation and product label • Removed adherence to wearable as a study procedure throughout the protocol.
3.0	10JAN2024	• The coordinated non-pharmacologic care patient log was changed from a daily log to a weekly log; similarly, the daily monitoring of BP was changed to weekly for those receiving coordinated non-pharmacologic care • Clarified throughout the protocol that the DSQ-PEM will be done twice at baseline: once on the day of the visit with a look back period of "past 7 days" and once the day after the visit with a look back period of "since last study visit". • Removed the following statement from Section 8.9.7 as it is not relevant: "At least one moderate symptom (severity ≥ 2, frequency ≥ 2) on the DSQ-PEM will make a participant included in this cluster and will trigger other baseline assessments listed below." • Reworded the statement in Section 8.9.10 to indicate that autonomic function tests will be completed at specific site (not select sites): "Additional autonomic function testing will be completed at specific sites that have the capability to perform these tests." • Updated Section 14.7 to present the information on Ivabradine 2.5 mg since the 2.5 mg strength (from Europe) will be the investigational product that is used in this study. The 2.5 mg tablet will be encapsulated to form a capsule (tablet + filler). • Stool collection was inadvertently missing from the End of Intervention visit in the Appendix B Schedule of Procedures. It has now been added.

		• Added text to Section 8.4 Study Intervention Visit and footnotes to the appendix specific Schedule of Procedures to clarify procedures that will be completed to determine dosing. • Added specification regarding medical history: At every clinic visit after screening visit, changes in medical history including SARS-CoV-2 infection since last visit will be collected. • Added clarification that the CBC labs will be "with differential". • Added concomitant medications review to each study visit and the early termination visit.
4.0	19SEP2024	• Clarified that HR measurements during the Active Stand Test will be evaluated as a secondary endpoint and BP measurements during Active Stand Test will be evaluated as an exploratory endpoint. • Moved wearable device measuring accelerometry (step count and HR) from secondary outcome measure to exploratory outcome measure. • Updated the description of the exploratory endpoint for autonomic function tests: *Change of quantitative autonomic symptoms score (QASAT) or sub-scores from baseline to EOI.* • Added specification for orthostatic hypotension: decline of ≥ 20 mmHg of systolic or ≥ 10 mmHg of diastolic blood pressure after 3 minutes of standing. • Removed specific mention of the medical history eCRF in Section 9.1.2 since the forms are still under production. • Updated Table 3. Sample size and Power Calculation for the Primary Outcome, Change of OHQ/OIQ Composite Score, from Baseline to EOI with 0.05 type I Error in Appendix A. • Removed 'Lactating and breast-feeding women' from Appendix B exclusion criteria since it is already covered under the master protocol exclusion criteria. • Updated Section 5.1 to indicate that oral contraception alone is considered an effective method of contraception. • Updated Section 10.3 regarding populations for analysis: Population for the primary efficacy analysis will be based on an intention-to-treat (ITT) population*, consisting of all randomized participants*. The population for safety analyses *will consist of all randomized participants who receive the randomized study intervention or control.* • Updated Section 10.4.5 regarding planned interim analysis: *Interim efficacy or futility analyses are not planned.* This protocol does not have planned early stopping rules for efficacy *nor for futility*. • Added the following statement to Section 10.4.7 Planned Test-Retest Reliability Analyses: The data from the first 50 subjects

		with 'No Change' status in the Patient Global Impression of Change will be used for the test-retest reliability analyses. • As requested by DSMB, updated Section 8.9.11 regarding the PASC Symptom Questionnaire to state that two questions, PGIC and PGIS, are included in the PASC questionnaire. • Added the specification regarding medical history: If medical records are not available, they are not required. History and physical examination could be performed at screening/baseline visit. • Updated the COMPASS-31 score inclusion criteria in the master protocol to score above 20, changed the COMPASS-31 score inclusion criteria for Appendix A from >40 to >25, and removed the COMPASS-31 score inclusion criteria from Appendix B. • Moved master level exclusion criteria 'Known atrial fibrillation or significant cardiac arrhythmia' to Appendix B specific exclusion criteria. • Moved master level exclusion criteria 'History of significant hypercoagulability disorders' to Appendix A specific exclusion criteria with updated wording 'History of significant hypercoagulability disorders that could lead to high risk of thrombosis on IVIG therapy, including significant thrombotic events after the acute phase of COVID-19 and/or within 6 months of enrollment' • Deleted the master level exclusion criteria: 'Active or recent thrombosis' • Removed master level exclusion criteria regarding beta-blockers and calcium-channel blockers. • Updated specification regarding re-enrollment: 'Patients who are a part of observational studies (e.g. imaging) may be included in this trial. Participants may enroll in another interventional the trial or re-enroll in this trial for a different study intervention appendix 30 days after end of study (Follow-up visit).' • Updated the orthostatic tachycardia definition in Appendix A inclusion criteria to allow for history of documented increase of 30 bpm or more from an active stand test or tilt table test without orthostatic hypotension in the past 1 year AND standing HR above 100 bpm at the Screening/Baseline visit active stand test. • Updated the orthostatic tachycardia definition in Appendix B inclusion criteria to allow for history of documented increase of 30 bpm or more from either an active stand test or tilt table test without orthostatic hypotension in the past 1 year AND

		standing HR above 100 bpm at the Screening/Baseline visit active stand test. • Added concomitant use of Paxlovid as an exclusion criterion for Appendix B since Paxlovid and ivabradine have a strong drug interaction related to CYP 3A4 and thus should not be given together. • Removed the following exclusion criteria from Appendix B: "Use of the following medications: clonidine, tizanidine, amphetamines, and serotonin and norepinephrine reuptake inhibitors (SNRIs) with the exception of modafinil." • Updated exclusion criteria regarding potassium-depleting diuretic in Appendix B: If potassium level is normal at the baseline CMP, participant may continue taking it during the study. • Updated the concomitant medication section of Appendix B regarding the use of beta-blockers and calcium channel blockers: *Participants on beta-blockers or calcium channel blockers will be requested to pause their medication 2 days prior to the active stand test at Screening/Baseline visit. This is not mandatory, but highly recommended if the participant is willing. Depending on the heart rate at the Baseline visit, the treating physician may ask the participant to either continue (HR > 60 bpm) or hold (HR < 60 bpm) their beta-blocker or calcium channel blocker during the study. The same medications that are held at Screening/Baseline visit should also be held prior to the EOI clinic visit.* • Updated start of intervention from Day 0 to Day 1 throughout protocol • Updated the clinic visit windows from weeks to days in the master schedule of procedures as well as the Appendix A and B schedule of procedures. This allows for a more accurate representation of the window. • Added footnotes to the master schedule of procedures to clarify that the OHQ/OIQ completed at Baseline visit will be used to determine eligibility, eligibility assessments (active stand test, OHQ/OIQ, and labs) should be completed prior to randomization, and some of the eligibility baseline assessments (active stand test and labs), can be performed at the Screening visit if the participant comes in-person for Screening.
5.0	03SEP2025	• Section 1.2 (Schema) Figure 1: Day 0 was updated to Day 1. • Section 5.1 (Inclusion Criteria) was revised to increase the enrollment cap of participants with suspected or probable SARS-CoV-2 infection from 10% to 20%.

		• Section 10.4.1 (Analysis of the Primary Endpoint): The statistical analysis description was revised by replacing “linear regression” with a broader “regression-based approach • Section 10.4.4 (Missing Data) was revised to remove the statement that no imputation would be performed and instead clarified that prespecified, model-based or principled approaches may be used to mitigate missingness, with full details to be provided in the SAP. • Section 10.4.6 (Planned Subgroup Analyses) was removed. • Section 10.4.8 (Planned Sample Size and Power Re-estimation) was removed to reflect that this activity will not be conducted for the study.

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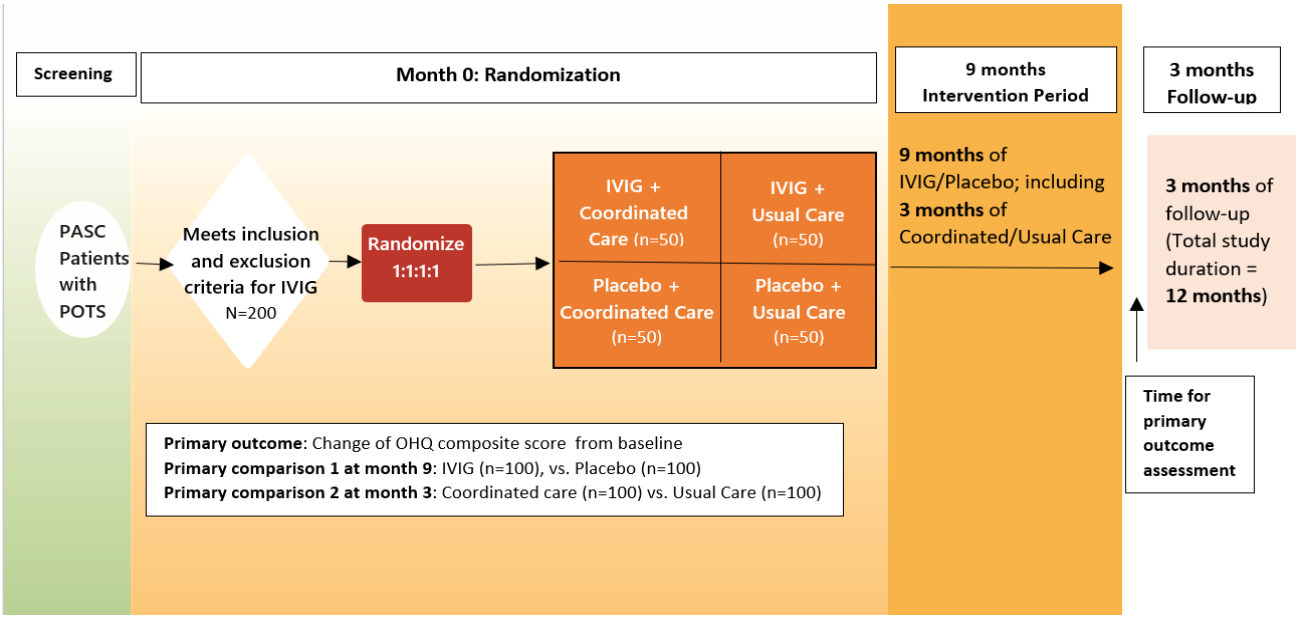
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13 APPENDIX A (IVIG)

13.1 STUDY DESIGN

Figure 2. Study Schema for Appendix A (IVIG)



*In this factorial design, participants will be randomized 1:1:1:1 to the combination of study drug /placebo and coordinated/usual care.

As depicted in **Figure 2**, approximately 200 participants will be enrolled in Appendix A (IVIG), based on eligibility criteria.

In the IVIG appendix, randomization will be implemented with 1:1:1:1 allocation among the combination of IVIG/placebo and coordinated/ usual non-pharmacologic care. All participants will receive IVIG or placebo for 9 months (36 weeks) with a follow-up period for an additional 3 months (total study duration for 12 months).

All participants will receive coordinated non-pharmacologic care or usual non-pharmacologic care (control) for a duration of 3 months. This will be concurrent with scheduled IVIG (or placebo) administration. coordinated non-pharmacologic care involves volume expansion through high salt diet (6-10 grams of salt), water intake (at least 2 liters per day), abdominal binder, exercise/rehabilitation, feedback, weekly monitoring of BP, motivation, education, and assisted care through a care coordinator.

The care coordinator will call each patient weekly during the 3-month non-pharmacologic intervention period to provide education and support on lifestyle modifications. Patients will be asked to maintain a weekly log of non-pharmacological care (average dietary intake of salt and water, physical

rehabilitation) and provide a summary to the care-coordinators during the weekly calls. Further details of the non-pharmacologic intervention are provided in Section 6.6 and contained in the MOP.

Skin biopsies will be completed for all participants. In addition, participants enrolled in sites that are selected for autonomic function tests will be required to complete the other autonomic function tests as per the platform protocol schedule of procedures.

13.2 INTERVENTION RATIONALE

IVIG is approved by the FDA for the treatment of various immune deficiency syndrome and autoimmune diseases. IVIG at high dose is thought to neutralize pathogenic autoantibodies and suppresses autoimmune response, and the use of high titers of anti-SARS-CoV-2 antibodies for the treatment of COVID-19 is authorized in the US. From the past experience of using IVIG on severe cases of POTS, IVIG can be associated with improved autonomic symptoms, as well as with overall symptom burden.[23-27] IVIG may also improve other, non-cardiovascular dysregulation and dysautonomia and a potential to provide disease-modifying effects. Therefore, IVIG is a promising option for patients with severe PASC presenting as post-COVID-19-POTS.

13.3 RISK ASSESSMENT

Serious and unexpected AEs may occur that have not been previously reported with IVIG use. The most common adverse reactions of IVIG reported in greater than 5% of subjects during previous clinical trials are headache, cough, injection site reaction, nausea, pharyngitis, urticaria, vomiting, fever, back pain, rash, chills, hypertension, and asthenia. Previously reported AEs were identified in the OCTA-088 study, which was a multi-dose, open-label, multi-center study in patients with primary immunodeficiency. Common AEs included abnormal hematologic and clinical chemistry findings. Following approval of IVIG, other AEs have been reported including thromboembolic events, such as myocardial infarction (MI), stroke, pulmonary embolism (PE), and deep vein thromboses (DVT). These may be serious depending on the site and type of thrombosis. Cases of aseptic meningitis have been reported; however, no fatal cases have been observed. Acute renal failure has been observed, but in most cases, it was mild, but may be serious in elderly patients, patients with diabetes, and patients with pre-existing renal disease. Hemolytic anemia /hemolysis has also been observed, but in most cases, it was mild and self-limited.[28]

The safety of IVIG for use in pregnancy and during lactation has not been established in controlled clinical trials. Clinical experience with immunoglobulins suggests that no harmful effects on the course of pregnancy, or on the fetus and the neonate are to be expected. Immunoglobulins are excreted into the milk and may contribute to the transfer of protective antibodies to the neonate.

Though side effects related to the pre- and post-medications (loratadine and acetaminophen) are rare, they can occur. Common adverse reactions of loratadine are headache, somnolence, fatigue, and dry mouth.[29] Common adverse reactions of acetaminophen are nausea, stomach pain, headache, hoarseness, loss of appetite, itching, rash, dark urine, clay-colored stools and swelling of the face, throat, tongue or limbs.

In a few cases, participants in this study may require steroids as pre-medication. General risks associated with steroids are increased appetite, acne, rapid mood swings, muscle weakness, and delayed wound healing.

13.4 ADDITIONAL APPENDIX-LEVEL INCLUSION CRITERIA

- Abnormal active standing test defined as presence of orthostatic tachycardia and experiencing orthostatic symptoms. Orthostatic tachycardia defined as:
    - Increase of 30 beats per minute (bpm) or more in HR within 10 minutes upon standing without orthostatic hypotension (decline of ≥ 20 mmHg of systolic or ≥ 10 mmHg of diastolic blood pressure after 3 minutes of standing)

      OR
    - History of documented increase of 30 bpm or more from an active stand test or tilt table test without orthostatic hypotension in the past 1 year AND standing HR above 100 bpm at the Screening/Baseline visit active stand test.
- COMPASS-31 Score > 25

13.5 ADDITIONAL APPENDIX-LEVEL EXCLUSION CRITERIA

- Current or previous IVIG treatment
- Contraindication to intravenous immunoglobulin.
- Known allergic reactions to blood products including IVIG and/or subcutaneous immunoglobulin (SCIG), such as history of clinically relevant hemolysis after IVIG infusion, aseptic meningitis, recurrent severe headache, hypersensitivity, severe generalized or severe local skin reactions
- Selective IgA deficiency
- Current and recent (within 5 half-lives) use of high-dose corticosteroids (for example for prior solid organ transplant), omalizumab, anti-TNF-alpha inhibitors
- Use of immunosuppressants such as Plaquenil, or low-dose steroid (prednisone, no more than 10mg a day) will be excluded unless the participant is on stable (>4 weeks) dose
- History of significant hypercoagulability disorders that could lead to high risk of thrombosis on IVIG therapy, including significant thrombotic events after the acute phase of COVID-19 and/or within 6 months of enrollment
- Veins that are not viable for infusions
- Not willing to adhere to dosing schedule for IVIG infusions for 9 months

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.

There is clinical evidence of an association between the administration of immunoglobulins and thromboembolic events such as MI, PE, and DVT. Therefore, caution should be exercised when administering IVIG.

Most IVIG adverse effects are mild and are related to the rate of infusion. Therefore, IVIG should be started at a slow rate of infusion and gradually increased according to patient tolerance. Patients predisposed to renal failure include patients with any degree of pre-existing renal insufficiency, diabetes mellitus, age greater than 65, volume depletion, sepsis, paraproteinemia, or patients receiving known nephrotoxic drugs. Especially in such patients, IVIG products should be administered at the minimum concentration available and the minimum rate of infusion practicable.

As with other IVIG formulations, this product is prepared from large pools of human plasma, which may contain the causative agents of hepatitis and other viral diseases. The risk that such products will transmit an infectious agent has been reduced by screening plasma donors for prior exposure to certain viruses, by testing for the presence of certain current virus infections, and by inactivating and/or removing certain viruses.

IgA deficient patients with antibodies against IgA are at greater risk of developing severe hypersensitivity and anaphylactic reactions.

As recommended in good clinical practice, epinephrine should be administered for management of anaphylaxis. Instructions will be given to participants to understand signs of allergic reactions and when to seek medical attention for possible allergic reactions.

Hyperproteinemia, with resultant changes in serum viscosity and electrolyte imbalances may occur in patients receiving IVIG therapy.

Aseptic Meningitis Syndrome (AMS) has been reported with IVIG treatments, especially with high doses or rapid infusion.

Hemolysis, either intravascular or due to enhanced red blood cell sequestration, can develop subsequent to IVIG treatments. Risk factors include high doses and non-O blood group. Therefore, it is advised to monitor for hemolysis and hemolytic anemia.

It is advised to monitor for pulmonary adverse reactions (transfusion related acute lung injury) and volume overload.[30]

The pre- and post-medications (loratadine and acetaminophen) should be used with caution in participants with decreased renal or liver function. These products are not to be used if the participant has had any allergic reactions to these ingredients in the past.[29] These medications will also be considered for participants who experience infusion related reactions.

13.6 CONCOMITANT MEDICATIONS

No medications or standard treatments for POTS should be withheld from study participants.

Participants who are already on stable (>4 weeks) doses of immunosuppressants as Plaquenil, methotrexate, and low-dose steroid (prednisone, no more than 10mg a day) will be allowed to continue the medication throughout the study.

During the study, initiation of steroids will not be allowed for more than 4 weeks, unless it is being used a pre-medication for IVIG treatment.

13.7 IVIG INFORMATION

Several formulations of IVIG have been approved by the FDA for use in various conditions including immune thrombocytopenic purpura, primary immunodeficiency, secondary immunodeficiency, Kawasaki disease, and prevention of graft versus host disease.

The FDA has not approved for the treatment of POTS or COVID-19. However, a number of case reports show improvement with immune modulating therapy, particularly IVIG, in patients with various post-COVID conditions

13.7.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

IVIG is a sterile liquid preparation of highly purified immunoglobulin G (IgG) derived from large pools of human plasma. Each mL contains 100 mg of protein, of which ≥96% is IgG. a sterile liquid preparation of highly purified IgG derived from large pools of human plasma.

IVIG will be supplied as a sterile solution for injection supplied in 20 g (200 mL) single-use, tamper evident vials.

13.7.2 DRUG DISPENSING, STORAGE, AND STABILITY

IVIG may be stored for 36 months at 2-8°C (36-46°F) from the date of manufacture, and it may be stored at temperatures not to exceed 25°C (77°F) for up to 6 months anytime during the 36 month shelf life, after which the product must be immediately used or discarded.[30]

13.7.3 DOSING AND ADMINISTRATION

IVIG (or placebo) will be administered intravenously by medical professional at the site. Double-blind will be maintained. The dose will be a **total of 2 g/kg every month**. The dosing frequency of 0.5 g/kg once a week is strongly suggested; however, the frequency can be modified based on individual site preference. There will be a dose cap at 80 kg (i.e. if weight > 80 kg, participant will be dosed at the 80 kg level).

The proposed dose (2 g/kg monthly) is evidence-based medicine. The dose is recommended for idiopathic thrombocytopenic purpura and chronic inflammatory demyelinating neuropathy.[31] There is no plan to vary the IVIG dose since the proposed dose is evidence-based medicine, and there is no evidence that the higher or lower dose is superior over the proposed dose. The same dose was also used in anecdotal studies for long COVID patients with dysautonomia and small fiber neuropathy.[32,33]

To reduce the occurrence of side effects and to enhance tolerability, fluids and pre- and post-meds are strongly encouraged. Therefore, 500 mL fluids (0.9% NaCl 500 mL IV), acetaminophen 650 mg, and loratadine 10 mg may be given as per PI discretion 30 minutes before IVIG (or placebo) infusion and after infusion. Some patients may require stronger pre- and post-medication (steroids, ketorolac) or additional fluids.

To ensure participant safety and monitor for adverse events, HR, BP, temperature, respiratory rate and oxygen saturation will be measured pre-, during, and post-infusion. Participants will be monitored for 30 minutes after receiving the infusion.

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

13.7.4 RATIONALE FOR SELECTION OF DOSE

Pre-clinical studies:

IVIG supplies a broad spectrum of opsonic and neutralizing IgG antibodies against bacterial, viral, parasitic, and mycoplasma agents, and their toxins. Immunoglobulins are fractionated blood products made from pooled human plasma. Immunoglobulins are endogenous proteins produced by B lymphocyte cells.

Pharmacokinetics

Following intravenous infusion, IVIG products show a biphasic decay curve. The initial (α) phase is characterized by an immediate post-infusion peak in serum IgG and is followed by rapid decay due to equilibration between the plasma and extravascular fluid compartments. The second (β) phase is characterized by a slower and constant rate of decay. The commonly cited “normal” half-life of 18 to 25 days is based on studies in which tiny quantities of radiolabeled IgG are injected into healthy individuals. When radiolabeled IgG was injected into patients with hypogammaglobulinemia or agammaglobulinemia, highly variable half-lives ranging from 12 to 40 days were observed. In other radiolabeled studies, high serum concentrations of IgG and hypermetabolism associated with fever and infection have been seen to coincide with a shortened IgG half-life.

In contrast, pharmacokinetic studies in immunodeficient patients are based on the decline of IgG concentrations following infusion of large quantities of immune globulin. In such studies, investigators have reported uniformly prolonged half-lives of 26 to 35 days.[34]

Clinical studies:

Human IVIG preparations for intravenous or subcutaneous administration were approved for number of indications.[35] FDA approved IVIG for diseases such as primary humoral immunodeficiencies, prevention of infections in lymphocyte depleted populations (B-cell lymphoma, bone marrow transplants, pediatric HIV), demyelinating, and autoimmune diseases. The recommended dose of IVIG, 1 to 2 gm/kg given over one to 5 days, is administered when treating idiopathic thrombocytopenic purpura or other autoimmune related conditions. For chronic inflammatory demyelinating neuropathy (CIDP), the standard dose is 0.4 g/kg/day for 5 days (total dose 2 gram/kg), but in relapsing patients this dose may need to be repeated every 2-8 weeks to maintain improvement.

IVIG is a complex therapy and can lead to a number of adverse events. Most adverse reactions to IVIG are infusion rate related and mild. The most common are headaches (16%), fever (7%), hypertension (5%), chills (3%) and nausea (3%).[31]

The Immune Globulin Intravenous (Human), 10% Caprylate/Chromatography Purified CIDP Efficacy, also known as the ICE study, was a multi-center, randomized, double-blind, placebo-controlled trial conducted with Gammaked IVIG.[36] This study included 2 separately randomized periods to assess whether Gammaked IVIG was more effective than placebo for the treatment of CIDP (assessed in the Efficacy Period for up to 24 weeks) and whether long-term administration of Gammaked IVIG could maintain long-term benefit (assessed in the 24 week randomized withdrawal period). The Inflammatory Neuropathy Cause and Treatment (INCAT) was used to assess functional disability of both upper and lower extremities in demyelinating polyneuropathy. More subjects with CIDP responded to Gammaked: 28 of 59 subjects (47.5%) responded to Gammaked compared with 13 of 58 subjects (22.4%) administered placebo (25% difference; 95% CI 7%-43%; p=0.006).

A randomized, double-blind, placebo-controlled, cross-over withdrawal study was conducted to evaluate the efficacy and safety/tolerability of Gammagard Liquid in adult subjects (N=44) with multifocal motor neuropathy.[37] The study examined grip strength in the more affected hand (measured with dynamometer), and Guy's Neurological Disability Scale (GNDS) [upper limb part 6 subsection]. The Overall Disability Sum Score (ODSS) changed by -7.14% during placebo (indicating worsening of disability) and by -1.11% (indicating minimal change in disability) during treatment with Gammagard liquid. For this specific analysis of ODSS, lower scores represented more disability. With the dominant hand, subjects required 17% longer to complete the 9-hole peg test (a measure of dexterity) at the end of the placebo period, compared with baseline. By contrast, at the end of the Gammagard liquid treatment period, participants required 1.2% longer to complete the 9-hole peg test for the dominant hand compared with baseline. With the non-dominant hand, subjects required 33% longer to complete the 9-hole peg test at the end of the placebo period and 6.7% longer at the end of the Gammagard liquid treatment period, compared with baseline.

A randomized, double-blind, multi-center, investigator-initiated study compared IVIG (Aventis Behring LLC, King of Prussia, PA) with placebo 5% albumin.[38] On Days 1, 2, and 21, IVIG (1 g/kg) or placebo was given. The primary outcome measure was the change in muscle strength from baseline to Day 42, using the average muscle score (AMS). Secondary outcome measures included change from baseline AMS at Days 10 and 21, the Hughes' functional disability scale, forced vital capacity (FVC), and nerve conduction

studies (NCS) of 4 motor nerves (median, ulnar, peroneal, and tibial). Total 33 patients were randomized. IVIG improved strength in patients with untreated CIDP by Day 10 with continued benefit through Day 42.

No randomized clinical trial has been performed to study IVIG in patients with PASC. Case studies of patients with PASC taking IVIG show improvements in symptom resolution.[28]

13.8 CONTROL INFORMATION

Normal saline given intravenously will be the control (placebo) product.

13.8.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The normal saline will be of similar formulation and appearance to IVIG.

13.8.2 CONTROL DISPENSING, STORAGE, AND STABILITY

Store at controlled room temperature 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

13.8.3 DOSING AND ADMINISTRATION

Control dosing and administration will occur according to Section 13.6.3 in order to maintain blinding.

13.9 EVENTS OF SPECIAL INTEREST

The following ESIs will be reported for IVIG:

- Aseptic meningitis
- Renal dysfunction (acute renal failure, osmotic nephrosis)
- Severe allergic reaction
- Thromboembolic events (DVT, PE, MI, ischemic stroke or other arterial thromboembolism)
- Acute hypertension greater than 180/110 (involving emergency visit or hospitalization)
- Episodes of anaphylaxis
- Episodes of other allergic reactions (rash, urticaria, shortness of breath, wheezing, tongue or lip swelling, injection site swelling and redness)
- Episodes of transfusion reactions (fever, chills, rigors)
- Episodes of acute renal failure unrelated to co-morbidities
- Episodes of aseptic meningitis
- Episodes of hemolytic anemia
- Episodes of acute lung injury with transfusion
- Episodes of thrombosis
- Any death

- Any other adverse reaction that resulted in the investigator or participant pausing or stopping study drug

13.10 ADDITIONAL APPENDIX-LEVEL HALTING AND STOPPING CRITERIA

1) Study drug infusion rate should be decreased by 50% if:
    a. The participant has a concerning increase in symptoms of fatigue, aching, shortness of breath, nausea, headache, or substantial change in HR or BP or oxygen saturation during the infusion that is determined by the investigator to be due to study drug but not representing an allergic reaction (but not representing an allergic reaction, in which case study drug should be interrupted or discontinued).

2) Study drug infusion should be interrupted if:
    a. The participant requests that the study drug be paused or stopped
    b. The investigator believes that for safety or tolerability reasons it is in the best interest of the participant to pause or stop study drug, for example for worsened shortness of breath and/or oxygen desaturation
    c. The participant develops a suspected allergic reaction; including new rash, new or worsened shortness of breath, or transfusion reaction during the infusion that is suspected to be related to the study medication

3) Redosing of study drug (subsequent weekly study drug dosing) should be interrupted (paused) if:
    a. The participant requests to interrupt study drug
    b. The investigator believes that, for safety reasons or tolerability reasons, it is in the best interest of the participant to interrupt study drug
    c. The participant develops any SUSAR
    d. The participant develops a suspected allergic or transfusion reaction that may be related to the study medication

13.11 STATISTICAL CONSIDERATIONS (APPENDIX-SPECIFIC)

Power calculation is provided for this IVIG appendix is based on a total of 200 enrolled participants. The power is 86% to detect 1-unit difference between 2 groups in change of OHQ/OIQ composite score if the missing data rate is 25% with a total sample size of 200. Table 3 provides variation of this assumption for a total sample size of 200 with corresponding powers for the primary endpoint.

We assumed that the missing data rate to range from 20% to 25% at month 9 based on the observed missing date of 30% at one year in the longitudinal study in POTS subjects.[39] The missing data rate of 25% is more likely in the trial with IVIG because of 9-month duration of intervention and the nature of intervention via injection

Table 3. Sample Size and Power Calculation for the Primary Outcome, Change of OHQ/OIQ Composite Score, from Baseline to EOI with 0.05 Type I Error

Sample size	Equal or Unequal Group Missing data rate(s)	Total or group Sample size(s) without missing data	Clinically Significant Difference	Equal or Unequal Group Standard Deviation(s)	Power
200	(15%, 20%)	(76, 72)	1.0	2.0	85.5%
200	(15%, 20%)	(76, 72)	1.0	2.1	82.0%
200	(15%, 20%)	(76, 72)	1.0	(2.0, 2.1)	83.7%
200	(15%, 20%)	(76, 72)	1.0	(2.0, 2.2)	81.8%
200	20%	160	1.0	2.0	88.2%
200	**25%**	**150**	**1.0**	**2.0**	**86.0%**
200	20%	160	1.0	2.1	84.9%
200	25%	150	1.0	2.1	82.6%
200	(25%, 30%)	(75, 70)	1.0	2.0	84.8%
200	(25%, 30%)	(75, 70)	1.0	2.1	81.2%
200	(25%, 30%)	(75, 70)	1.0	(2.0, 2.1)	82.9%
200	(25%, 30%)	(75, 70)	1.0	(2.0, 2.2)	81.0%

For the secondary outcome, COMPASS-31, the power is 86% with a total sample size of 200 to detect 10-unit difference in change of COMPASS-31 from baseline to 9-month follow-up between the 2 groups assuming SD of 20 (equivalent to effect size of 0.5) and 25% missing data rate.

13.12 SCHEDULE OF PROCEDURES

Study Intervention Period Schedule of Activities for Appendix A; IVIG

	Study Intervention Period			
	Start of Study Intervention	**3 Month Clinic Visit**	**6 Month Clinic Visit**	**End of Intervention (9 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 84 ± 7 days (Day 77 to Day 91)**	**Day 168 ± 7 days (Day 161 to Day 175)**	**Day 252 ± 7 days (Day 245 to Day 259)**
Concomitant Medications		X	X	X
Pre and Post-Dose Administration[1]	X	X	X	
Study Drug Administration[1,5]	X	X	X	
Coordinated or Usual Non-pharmacologic Care[2]	X	X		
OHQ/OIQ		X	X	X
COMPASS-31		X	X	X
MAPS		X	X	X
VOSS		X	X	X
Active Stand Test		X	X	X
6-minute Walk Test		X	X	X
DSQ-PEM[3] (look back period “since last visit”)		X	X	X

	Study Intervention Period			
	Start of Study Intervention	**3 Month Clinic Visit**	**6 Month Clinic Visit**	**End of Intervention (9 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 84 ± 7 days (Day 77 to Day 91)**	**Day 168 ± 7 days (Day 161 to Day 175)**	**Day 252 ± 7 days (Day 245 to Day 259)**
PROMIS-29 + 2 Questionnaire		X	X	X
Skin Biopsy	Skin biopsy will be completed at baseline for all participants. If the skin biopsy shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI.			
Blood Collection[6]				X
PASC Symptom Questionnaire		X	X	X
Vital Signs monitoring[7]	X			
Stool collection				X
Autonomic function tests at specialized sites				X
Height and weight[8]	X			
Safety Assessment of AEs, including SAEs and ESIs[4]	X	X	X	X

Abbreviations: EOI – End of Intervention, QOL – Quality of Life; PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ- Orthostatic intolerance questionnaire; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1] Pre-and post-dose administration and study drug administration will be done throughout the study intervention period

[2] Only applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only.

[3] DSQ-PEM questionnaire will be a via phone or electronic survey within one day after strenuous in-person study visits.

[4] Non-serious AEs will be reportable from start of study drug dosing to end of study drug administration.

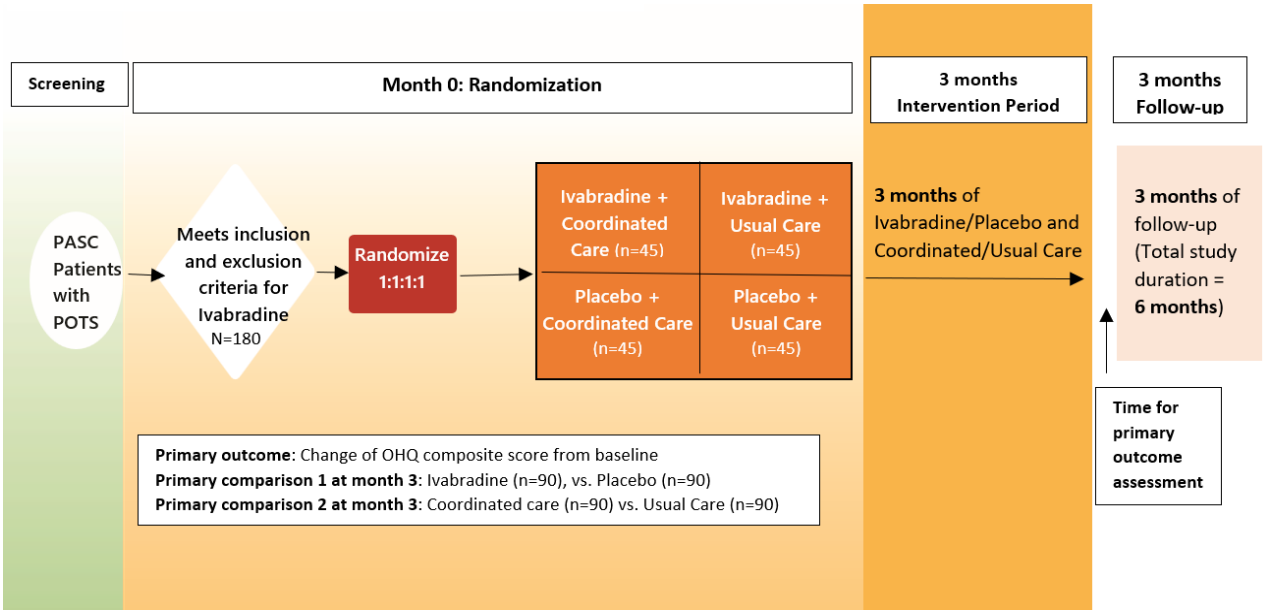
[5] The dosing frequency of 0.5 g/kg once a week is strongly suggested; however, the frequency can be modified based on individual site preference. The dose will be a **total of 2 g/kg every month** for 9 months.

[6] Blood may be collected at any time as needed based on PI discretion and adverse events.
[7] Vital signs monitoring will be done pre-, during, and post-infusion to ensure participant safety and monitor for adverse events.
[8] Weight will be captured at each infusion visit.

14 APPENDIX B (IVABRADINE)

14.1 STUDY DESIGN

Figure 3. Study Schema for Appendix B (Ivabradine)



*In this factorial design, participants will be randomized 1:1:1:1 to the combination of study drug/placebo and coordinated/usual care.

As depicted in **Figure 3**, approximately 180 participants will be enrolled in Appendix B (Ivabradine), based on eligibility criteria.

In the ivabradine study, randomization will be implemented with 1:1:1:1 allocation among the combinations of ivabradine/placebo and coordinated/usual care. All participants receive either ivabradine or placebo for 3 months (12 weeks) with a follow-up period for an additional 3 months (total study duration of 6 months).

All participants will receive coordinated non-pharmacologic care or usual non-pharmacologic care (control) for a duration of 3 months. This will be concurrent while taking ivabradine (or placebo). coordinated non-pharmacologic care involves volume expansion through high salt diet (6-10 grams of salt), water intake (at least 2 liters per day), abdominal binder, exercise/rehabilitation, weekly monitoring of BP, motivation, education, and assisted care through care coordinator.

The care coordinator will call each patient weekly during the 3-month non-pharmacologic intervention period to provide education and support on lifestyle modifications. Patients will be asked to maintain a weekly log of non-pharmacological care (average dietary intake of salt and water, exercise regimen) and

provide a summary to the care-coordinators during the weekly calls. Further details of the non-pharmacologic intervention are provided in Section 6.6 and contained in the MOP.

Autonomic function tests are optional for this appendix but recommended as per the platform protocol schedule of procedures.

14.2 INTERVENTION RATIONALE

Ivabradine is a medication used to treat chronic heart failure with elevated HR. It reduces HR without reducing contractility or BP. It has the greatest activity and most potent effect when the HR is elevated. It increases coronary flow reserve and collateral perfusion, promoting the development of coronary collaterals and improving endothelial function. In addition, it decreases norepinephrine levels in patients with POTS. Ivabradine has been successfully used to treat symptoms such as tachycardia and POTS in a small, randomized trial, and its use has been associated with improvement in symptoms in patients with PASC and POTS.[34]

14.3 RISK ASSESSMENT

Serious and unexpected AEs may occur that have not been previously reported with ivabradine use. Previously reported AEs were identified in the SHIFT study, which was a randomized, placebo-controlled trial in patients with systolic heart failure. Common AEs included (incidence ≥ 1.0% higher on ivabradine than placebo and occurring in > 1% on ivabradine) included bradycardia, hypertension, atrial fibrillation, and phosphenes (visual brightness). Following approval of ivabradine, the following AEs have been reported: syncope, hypotension, torsade de pointes, ventricular fibrillation, ventricular tachycardia, angioedema, erythema, rash, pruritus, urticaria, vertigo, and diplopia, and visual impairment.

No ivabradine 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. The risk/benefit of treatment with ivabradine for PASC has not been established in pregnant women.

14.4 ADDITIONAL APPENDIX-LEVEL INCLUSION CRITERIA

- Abnormal active standing test defined as presence of orthostatic tachycardia and experiencing orthostatic symptoms. Orthostatic tachycardia defined as:
    - Increase of 30 beats per minute (bpm) or more in HR within 10 minutes upon standing without orthostatic hypotension (decline of ≥ 20 mmHg of systolic or ≥ 10 mmHg of diastolic blood pressure after 3 minutes of standing)

      OR
    - History of documented increase of 30 bpm or more from an active stand test or tilt table test without orthostatic hypotension in the past 1 year AND standing HR above 100 bpm at the Screening/Baseline visit active stand test.

14.5 ADDITIONAL APPENDIX-LEVEL EXCLUSION CRITERIA

- A person of child-bearing potential who is not taking effective contraception
- Use of midodrine, pyridostigmine, fludrocortisone, and guanfacine will be excluded unless participant is on a stable dose (>4 weeks). Participants on stable doses will be allowed to continue the medication throughout the study.
- Combination with verapamil or diltiazem which are moderate CYP3A4 inhibitors with heart rate reducing properties
- Severe hepatic impairment
- Use of drugs known to prolong the QT-interval (e.g., quinidine, disopyramide, bepridil, sotalol, amiodarone, pimozide, ziprasidone, sertindole, mefloquine, halofantrine, pentamidine
- Concomitant use of digoxin or Paxlovid
- Known history of atrial fibrillation or significant cardiac arrhythmia not due to reversible cause
- Participants who are pacemaker dependent
- Patients with hypokalemia (serum K+<3.5 mEq/L)
- Patients taking potassium-depleting diuretics, unless potassium level is normal at the baseline CMP, in which case participants may continue taking it during the study.
- A history of congenital or acquired long QT syndrome, with or without torsade de pointes
- Patients with high degree AV block such as Type 2 AV block, Mobitz II

14.5.1 PRECAUTIONS

The healthcare provider should consult other appropriate resources such as the prescribing information for comprehensive information on dosing and monitoring.

Initiation of medications that inhibit or induce CYP3A may increase or decrease concentrations of ivabradine, 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 ivabradine
- Loss of therapeutic effect of ivabradine

Concomitant administration of ivabradine with negative chronotropes (such as calcium channel blockers and beta-blockers) increases the risk of bradycardia. Therefore, close monitoring of heart rate is required.

14.6 CONCOMITANT MEDICATIONS

Participants who are already on stable (>4 weeks) doses of midodrine, pyridostigmine, fludrocortisone, and guanfacine will be allowed to continue the medication throughout the study.

Participants on beta-blockers or calcium channel blockers will be requested to pause their medication 2 days prior to the active stand test at Screening/Baseline visit. This is not mandatory, but highly recommended if the participant is willing.

Depending on the heart rate at the Baseline visit, the treating physician may ask the participant to either continue (HR > 60 bpm) or hold (HR < 60 bpm) their beta-blocker or calcium channel blocker during the study.

The same medications that are held at Screening/Baseline visit should also be held prior to the EOI clinic visit.

During the study, initiation of the following medications will not be allowed for more than 2 weeks:

- Midodrine
- Pyridostigmine

14.7 IVABRADINE INFORMATION

Ivabradine is a hyperpolarization-activated cyclic nucleotide-gated channel blocker. In April 2015, the US FDA approved ivabradine for use in adult and pediatric patients to treat stable symptomatic heart failure with an ejection fraction of ≤35%. Currently, ivabradine is not FDA approved for the treatment of POTS or PASC.

14.7.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Ivabradine 2.5 mg capsules for oral administration, equivalent to 2.5 mg of ivabradine hydrochloride will be used. The capsule will consist of the 2.5 tablet plus fillers (microcrystalline cellulose).

The tablet contains the following inactive ingredients: betadex, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate. The film-coating contains hypromellose, lactose monohydrate, titanium dioxide, macrogol 4000, iron oxide red, iron oxide yellow, and iron oxide black.

14.7.2 DRUG DISPENSING, STORAGE, AND STABILITY

Store at controlled room temperature 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

14.7.3 DOSING AND ADMINISTRATION

The starting dose will be 5 mg twice a day, and the dose will be modified if needed at the 1-month clinic visit. At this visit, HR will be measured, and the dose will be modified as applicable. The maximum dose will be 7.5 mg twice daily if HR is > 90 bpm.

The table below provides the dosing of ivabradine based on HR.

Supine Resting HR 60-80	2.5 mg BID
Supine Resting HR >80	5 mg BID
Supine Resting HR >90	7.5 mg BID

*Resting HR should be measured 5 minutes after lying down

During the study intervention period, the study sites will contact participants once a week to monitor compliance with study drug administration.

Ivabradine tablets should generally be taken with food and the tablets should be swallowed whole, not chewed, broken, or crushed.

If the participant misses a dose of ivabradine 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.

Overdose (defined as an excess of 7.5 mg twice a day) may lead to severe and prolonged bradycardia. In the event of bradycardia with poor hemodynamic tolerance, temporary cardiac pacing may be required. Supportive treatment, including intravenous (IV) fluids, atropine, and intravenous beta-stimulating agents such as isoproterenol, may be considered.

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.

14.7.4 RATIONALE FOR SELECTION OF DOSE

Pre-clinical studies:
There was no evidence of carcinogenicity when mice and rats received ivabradine up to 104 weeks by

dietary administration. High doses in these studies were associated with mean ivabradine exposures of at least 37 times higher than the human exposure ($AUC_{0\text{-}24hr}$) at the maximum recommended human dose.

Clinical studies:
In the SHIFT trial, all subjects were initiated on ivabradine 5 mg (or matching placebo) twice daily and the dose was increased to 7.5 mg twice daily or decreased to 2.5 mg twice daily to maintain the resting HR between 50 and 60 bpm.

No pharmacokinetic differences have been observed in elderly (≥ 65 years) or very elderly (≥ 75 years) patients compared to the overall population. However, ivabradine has only been studied in a limited number of patients ≥ 75 years of age.

No dose adjustment is required in patients with mild or moderate hepatic impairment.

No dosage adjustment is required for patients with creatinine clearance 15 to 60 mL/min.

No randomized clinical trial has been performed to study ivabradine in patients with PASC. Case studies of patients with PASC taking ivabradine show improvements in symptom resolution.[40]

14.8 CONTROL INFORMATION

The control (placebo) oral tablets will be similar to the study drug, ivabradine.

14.8.1 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The placebo will be salmon-colored, film-coated tablets, that match the appearance of ivabradine.

The control packaging matches the packaging described in Section 14.7.1.

14.8.2 CONTROL DISPENSING, STORAGE, AND STABILITY

Store at controlled room temperature 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

14.8.3 DOSING AND ADMINISTRATION

Control dosing and administration will occur according to Section 14.7.3 in order to maintain blinding.

14.9 EVENTS OF SPECIAL INTEREST

The following ESIs will be reported for ivabradine:

- Bradycardia
- Hypertension
- Atrial fibrillation
- Luminous phenomena (phosphenes)

14.10 STATISTICAL CONSIDERATIONS (APPENDIX-SPECIFIC)

Power calculation is provided for this Ivabradine appendix is based on a total of 180 enrolled participants. The power is 86.5% to detect 1-unit difference between two groups in change of OHQ/OIQ composite score if the missing data rate is 15% with a total sample size of 180. Table 4 provides variation of this assumption for a total sample size of 180 with corresponding powers for the primary endpoint.

We assumed that the missing data rate to be 15% at month 9 based on the observed missing date of 30% at one year in the longitudinal study in POTS subjects.[39]

Table 4. Sample size and power calculation Power Calculation for the Primary Outcome, change of OHQ/OIQ composite score, from baseline to EOI with 0.05 type I error

Sample size	Equal or Unequal Group Missing data rate(s)	Total or group Sample size(s) without missing data	Clinically Significant Difference	Equal or Unequal Group Standard Deviation(s)	Power
180	**15%**	**153**	**1.0**	**2.0**	**86.5%**
180	20%	144	1.0	2.0	84.6%
180	15%	153	1.0	2.1	83.1%
180	20%	144	1.0	2.1	81.0%

For the secondary outcome, COMPASS-31, the power is 86.9% to detect 10-unit difference in change of COMPASS-31 from baseline to 3-month follow-up between the two groups assuming SD of 20 (equivalent to effect size of 0.5) and 15% missing data rate.

14.11 SCHEDULE OF PROCEDURES

Study Intervention Period Schedule of Activities for Appendix B: Ivabradine

	Study Intervention Period		
	Start of Study Intervention	**1 Month Clinic Visit**	**End of Intervention (3 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 28 ± 7 days (Day 21 to Day 35)**	**Day 84 – 7 days (Day 77 to Day 84)**
Concomitant Medications		X	X
Study Drug Administration[1]	X	X	
Coordinated Non-pharmacologic Care[2]	X	X	X
Weekly site calls[3]	X	X	
OHQ/OIQ Assessment		X	X
COMPASS-31		X	X
MAPS		X	X
Stool Collection			X
Active Stand Test and Vanderbilt Orthostatic Symptoms Score		X	X
6-minute Walk Test		X	X

	Study Intervention Period		
	Start of Study Intervention	**1 Month Clinic Visit**	**End of Intervention (3 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 28 ± 7 days (Day 21 to Day 35)**	**Day 84 – 7 days (Day 77 to Day 84)**
DSQ-PEM[4] (look back period "since last visit")		X	X
PROMIS-29 + 2 Questionnaire		X	X
Blood Collection[6]			X
PASC Symptom Questionnaire		X	X
Autonomic function tests at specific sites			X
Safety Assessment of AEs, including SAEs and ESIs	X	X	X
Vital signs monitoring[7]		X	
Adherence[5]	X	X	X

Abbreviations: EOI – End of Intervention, QOL – Quality of Life; PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ- Orthostatic intolerance questionnaire; AE – Adverse Events; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1]Study drug administration will occur every day during the intervention period

[2] Only applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only.

[3]Participants will receive weekly phone calls for the first 30 days of the study intervention period

[4] DSQ-PEM questionnaire will be via phone or electronic survey within one day after strenuous in-person study visits.

[5] Adherence to drug (pill count) will be documented.

[6] Blood collection may be collected at any time as needed based on PI discretion and adverse events.

[7] HR will be captured at the 1-month clinic visit to determine dosing.

A Platform Protocol for Evaluation of Interventions for Autonomic Dysfunction in Post-Acute Sequelae of SARS-CoV-2 Infection (PASC)

Protocol: RECOVER-AUTO (Appendix B Ivabradine)

STATISTICAL ANALYSIS PLAN (SAP)

Protocol Version 5.0

Protocol Version Date: 03-SEP-2025

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We, the undersigned, have read and approve of this SAP and agree on its content.

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List of Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
AST	Active Stand Test
BMI	Body Mass Index
CBFv	Cerebral Blood Flow Velocity
CI	Confidence Interval
COMPASS-31	Composite Autonomic Symptoms Score 31
COVID-19	Coronavirus Disease of 2019
DCRI	Duke Clinical Research Institute
DSMB	Data and Safety Monitoring Board
DSQ - PEM	DePaul Symptom Questionnaire Post Exertional Malaise
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOI	End of Intervention
ESI	Events of Special Interest
HUT	Head Up Tilt Test
IVAB	Ivabradine
MAPS	Malmo POTS Symptom Score
MedDRA	Medical Dictionary of Regulatory Activities
MITT	Modified Intention-To-Treat
OHQ	Orthostatic Hypotension Questionnaire
OIQ	Orthostatic Intolerance Questionnaire
OIDAS	Orthostatic Intolerance Daily Activity Scale
OISA	Orthostatic Intolerance Symptom Assessment
PASC	Post-acute Sequelae of SARS-CoV-2 Infection
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
PROs	Patient Reported Outcomes
PROMIS-29+2	Patient-Reported Outcomes Measurement Information System
PT	Preferred Term
Q1	25th Percentile (1st Quartile)
Q3	75th Percentile (3rd Quartile)
RECOVER	Researching COVID to Enhance Recovery
RSA	Respiratory Sinus Arrhythmia
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SE	Standard Error
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCD	Transcranial Doppler
VOSS	Vanderbilt Orthostatic Symptoms Score

1 STUDY AND DOCUMENT OVERVIEW

RECOVER-AUTONOMIC is a platform protocol evaluating treatment of interventions for Autonomic Dysfunction in Post Acute Sequelae of SARS-CoV-2 Infection (PASC). This is a prospective, multi-center, multi-arm, randomized, controlled platform trial in patients with previous suspected, probable, or confirmed SARS-CoV-2 infection, as defined by the Pan American Health Organization. Eligible subjects will have experienced autonomic dysfunction (Moderate, self-identified autonomic symptoms (defined as COMPASS-31 >20 and OHQ/OIQ question 1 score >2 for Appendix B) following a SARS-CoV-2 infection that has persisted for at least 12 weeks and is still present at the time of consent.

This document describes the planned statistical analyses that will be conducted at the completion of the RECOVER-AUTO study in Appendix B. Once the initial un-blinded data review has occurred, only the blinded statistics team members will be allowed to modify the SAP (see Appendix 1 for the list of blinded and un-blinded statisticians).

Approximately 180 participants will be enrolled in Appendix B. Within this study, participants will be assigned with equal probability to one of the factorial combinations based on two factors: (1) Ivabradine /placebo and (2) Coordinated non-pharmacologic care/usual non-pharmacologic care. The intervention period will be 3 months of both Ivabradine/placebo and 3 months of Coordinated non-pharmacologic care/usual non-pharmacologic care (concurrent with Ivabradine/placebo administration) with 3 additional months for follow-up. Coordinated non-pharmacologic care involves volume expansion through high salt diet (6-10 grams of salt), water intake (at least 2 liters [64 ounces] per day), abdominal binder, exercise/rehabilitation, feedback, weekly monitoring of BP, motivation, education, and assisted care through a care coordinator. The care coordinator will call each patient weekly during the 3 month non-pharmacologic intervention period to provide education and support on lifestyle modifications. Participants will be asked to maintain a weekly log of non-pharmacological care (average dietary intake of salt and water, physical rehabilitation) and provide a summary to the care-coordinators during the weekly calls.

Safety monitoring will be performed throughout the trial and reviewed by the Data and Safety Monitoring Board (DSMB). The DSMB established for the RECOVER project will have oversight responsibility for the study. In the event that the DSMB recommends to discontinue study activities, enrollment and intervention-specific activities will be temporarily suspended while the NIH and the study Co-Principal Investigators consider the DSMB recommendations prior to making decisions on study continuation or discontinuation.

The effectiveness of the intervention programs (treatments) will be evaluated using the primary endpoint, change of Orthostatic Hypotension Questionnaire (OHQ)/OIQ composite score. Our main interest is to compare the change in OHQ score from baseline to end of intervention (EOI) between the different treatment groups listed below where the EOI is at month 3 for both comparisons:

A. Month 3: Ivabradine versus placebo
B. Month 3: Coordinated non-pharmacologic care versus usual non-pharmacologic care

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective:

- To Evaluate the effect of study intervention versus control on orthostatic intolerance.

2.1.2 Secondary Objectives:

- To evaluate the effect of study intervention versus control on symptom-specific outcome measures.
- To describe the effect of study intervention versus control on performance-based and vital sign outcome measures.
- To evaluate the effect of study intervention versus control on quality of life.

2.1.3 Safety Objectives:

- To characterize the safety and tolerability of study interventions for treatment of PASC.

2.1.4 Exploratory Objectives:

- To evaluate the effect of study intervention versus control on autonomic function tests.
- To describe the effect of study intervention versus control on performance-based and vital sign outcome measures.
- To describe the effect of study intervention versus control on PASC symptoms.
- To evaluate the effect of study intervention versus control on relevant biomarkers.
- To describe the effect of study intervention versus control on performance-based symptom measures.
- Compare the effect of study intervention versus control on specific tracked measurements.

2.2 Endpoints

This section summarizes the study endpoints and implementation details.

2.2.1 Primary Endpoints

The change in Orthostatic Hypotension Questionnaire (OHQ)/OIQ composite score from baseline to end of intervention with the following two primary comparisons.

- Month 3: Ivabradine versus placebo.
- Month 3: Coordinated non-pharmacologic care versus usual non-pharmacologic care.

2.2.2 Secondary Endpoints

The same two comparisons as in the primary endpoint will be carried out based on the following defined secondary endpoints.

- The change in Composite Autonomic Symptoms Score (COMPASS-31) from baseline to end of intervention.
- The change in Malmo POTS Symptom Score (MAPS) from baseline to end of intervention.

- The change in heart rate change (Highest heart rate standing -Lowest supine heart rate) during Active Stand Test from baseline to end of intervention.
- The change in heart rate (Lowest supine heart rate) during Active Stand Test from baseline to end of intervention.
- The change in total distance walked in 6-minute Walk Test from baseline to end of intervention.
- The change in PROMIS-29 Physical Limitations Summary Score from baseline to end of intervention.

2.2.3 Safety Endpoints

- Proportion of participants who experience individual serious adverse event (SAEs) and the proportion who experience any one or more SAEs (from first invasive study procedure (through end of study).
- Incidence of SAEs leading to discontinuation.
- Incidence of events of special interest (ESIs)
  - Bradycardia
  - Hypertension
  - Atrial fibrillation
  - Luminous phenomena (phosphenes)

2.2.4 Exploratory Endpoints

- The change in heart rate at every minute (1 through 10) in Head-up tilt (HUT) test from baseline (lying down 10 minutes) to end of intervention.
- The change in systolic and diastolic blood pressure (at 1, 3, 5, and 10 minute) in Head-up tilt (HUT) test from baseline (lying down 10 minutes) to end of intervention.
- The change in end-tidal CO2 (at 1 through 10 minutes) in Head-up tilt (HUT) test from baseline (lying down 10 minutes) to end of intervention.
- The change in cerebral blood flow systolic and diastolic (1 through 10 minute) in Transcranial Doppler (TCD), if available (with heads up tilt test) from baseline (lying down 10 minutes) to the end of intervention.
- The change in mean blood pressure phase II late in Valsalva Maneuver (with pressure of 40 mmHg for 15 seconds) from baseline through end of intervention.
- The change in mean blood pressure phase IV minus start blood pressure in Valsalva Maneuver (with pressure of 40 mmHg for 15 seconds) from baseline through end of intervention.
- The change in sinus arrhythmia (max heart rate /min heart rate over phase IV) in Valsalva Maneuver (with pressure of 40 mmHg for 15 seconds) from baseline through end of intervention.
- The change in time (seconds) to return to baseline systolic blood pressure in Valsalva Maneuver (with pressure of 40 mmHg for 15 seconds) from baseline through end of intervention.
- The change in respiratory sinus arrhythmia (RSA) in deep breathing test from baseline through end of intervention.
- The change in Vanderbilt Orthostatic Symptoms Score (VOSS) from baseline through end of intervention.
- The change in each question in PASC Symptom Questionnaire from baseline through end of intervention.

- The change in systolic and diastolic blood pressure in Active Stand Test from baseline (supine blood pressure corresponding to the timepoint at lowest supine heart rate) to end of intervention.
- The change in systolic and diastolic blood pressure change in Active Stand Test from baseline (lowest supine blood pressure) to end of intervention.
- The change in average daily total step count from wearable devices from baseline to end of intervention.
- The change in average heart rate from wearable devices from baseline to end of intervention.
- The change in viral biomarkers from baseline through end of intervention.
- The change in autonomic function biomarkers from baseline through end of intervention.
- The change in immune biomarkers from baseline through end of intervention.
- The change in inflammatory biomarkers from baseline through end of intervention.
- The change in endothelial function biomarkers from baseline through end of intervention.
- The change in cognitive function (CCL11) from baseline through end of intervention.

3 STATISTICAL DESIGN

3.1 Power and Sample Size

Sample size is based on power for assessing the primary main effect of Ivabradine versus placebo using two-sided alpha = 0.05. The type I error is not adjusted to account for a second test of coordinated versus usual non-pharmacologic care. This is justified by the trial's exploratory purpose and the viewpoint that the tests are answering stand-alone research questions, allowing type-I error to be controlled marginally instead of jointly across two comparisons.

The power calculation assumes a total enrollment of 180 participants. With this sample size and an anticipated 15% missing data rate, the study has 86% power to detect 1-unit difference between two groups in the change of OHQ/OIQ composite score. Table 1 provides variations of these assumptions, all based on a total sample size of 180 participants.

Our power calculation is based on a clinically significant difference taken to be a one unit change in the OHQ/OIQ composite score. This is based on an OHQ-OIQ validation study which found the estimated minimally important difference (MID) to range from 0.8 to 1.0 (1). The assumption of standard deviation (SD) of 2 in the change of OHQ/OIQ composite score is based on the observed SDs of 2.07 (active arm) and 1.69 (control arm) in the change of OHQ/OIQ composite score within 7 days in a phase 3 randomized, placebo-controlled clinical trial on droxidopa for neurogenic orthostatic hypotension (2). The observed SD of OHQ/OIQ composite score in 48 POTS subjects is 1.68; this implies that a SD of 2 or 2.1 in the change of OHQ/OIQ composite score corresponds to a correlation between OHQ/OIQ composite score at baseline and the EOI of 0.3 or 0.2.

Table 1. Sample size and power calculation Power Calculation for the Primary Outcome, change of OHQ/OIQ composite score, from baseline to EOI with 0.05 type I error

Sample size	Equal or Unequal Group Missing data rate(s)	Total or group Sample size(s) without missing data	Clinically Significant Difference	Equal or Unequal Group Standard Deviation(s)	Power
180	**15%**	**153**	**1.0**	**2.0**	**86.5%**
180	20%	144	1.0	2.0	84.6%
180	15%	153	1.0	2.1	83.1%
180	20%	144	1.0	2.1	81.0%

For the secondary outcome, COMPASS-31, the power is 86.9% to detect 10-unit difference in change of COMPASS-31 from baseline to 3-month follow-up between the two groups assuming SD of 20 (equivalent to effect size of 0.5) and 15% missing data rate.

3.2 Randomization Methods

The study uses a 2 x 2 factorial design, for the study drug (Ivabradine) in combination with non-pharmacologic intervention. Participants will be randomly allocated (1:1:1:1) in the four treatment groups below:

1. Ivabradine + Coordinated Care
2. Ivabradine + Usual Care
3. Placebo + Coordinated Care
4. Placebo + Usual Care

3.3 Interim Analysis and Data Monitoring

Interim examination of clinical endpoints and key safety events will be performed at regular intervals during the course of the trial. An independent, NIH-appointed, DSMB will monitor participant safety and review performance of the trial. The primary objective of these interim analyses will be to ensure the safety of the participants enrolled in the trial. 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.

This protocol does not have planned early stopping rules for efficacy or futility because this is a Phase II trial. 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. Stopping early would also limit the collection of data that are critical for planning future trials in similar patient populations.

4 STUDY ASSESSMENTS AND DEFINITIONS

4.1 Study Assessments

All participants will receive Ivabradine or placebo for 3 months (18 weeks) with a follow-up period for an additional 3 months (total study duration for 6 months). All participants will receive coordinated non-pharmacologic care or usual non-pharmacologic care (control) for a duration of 3 months. This will be concurrent with scheduled Ivabradine (or placebo) administration. Patients will be asked to maintain a weekly log of non-pharmacological care (average dietary intake of salt and water, physical rehabilitation) and provide a summary to the care-coordinators during the weekly calls. Skin biopsies will be completed for all participants. In addition, participants enrolled in sites that are selected for autonomic function tests will be required to complete the other autonomic function tests as per the platform protocol schedule of procedures.

The collection schedule for selected key study data is summarized in Abbreviations: PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ- Orthostatic intolerance questionnaire; AE-Adverse Events; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1Some] of the eligibility baseline assessments (active stand test and labs), can be performed at the Screening visit if the participant comes in-person for Screening.
[2] Screening cannot be on the same day as the Baseline/Randomization visit.
[3] Eligibility assessments (active stand test, OHQ/OIQ, and labs) should be completed prior to randomization.
[4A] detailed schedule of activities for the Study Intervention Period is provided in each Appendix.
[5] Follow-up visit windows for each appendix:
Appendix A: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 336 (Day 322 to Day 350)
Appendix B: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 168 (Day 154 to Day 182)
[6] If medical Records are not available, they are not required. History and physical examination could be performed at screening/baseline visit. At every clinic visit after screening visit, changes in medical history including SARS-CoV-2 infection since last visit will be collected.
[7Only] applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only. During this time, a care coordinator will reach out to the participants weekly.
[8] The OHQ/OIQ completed at Baseline visit will be used to determine eligibility.
[9] The DSQ-PEM will be done twice for the baseline visit: once on the day of the visit with a look back period of “past 7 days” and once within one day after the visit with a look back period of “since last study visit (SLV)”. DSQ-PEM SLV will be administered via phone or electronic survey. The DSQ-PEM administered at every timepoint after baseline will be within one day after strenuous in-person study visits and will ask about the period “SLV”.
[10] Blood collection for safety parameters, biomarkers, and biospecimen for biorepository. Blood collection may also be collected as needed based on PI discretion and adverse events.
[11] Skin biopsy is only applicable to Appendix A: IVABRADINE. If the baseline skin biopsy shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET.
[12] It is required to wear the wearable at least 7 days before start of study drug intervention and 7 days before end of intervention visit. All other times, it is strongly encouraged.
[13] Adherence to drug (pill count) will be documented. Participants receiving oral study drugs to be taken at home will receive weekly phone calls for the first 30 days.

Table 2a (Platform protocol) and Table 2b (Appendix B).

Table 1a. Schedule of Study Procedures: Platform Protocol

	Screening[1]	**Baseline/ Randomization[2,3]**	**Study Intervention Period[4]**	**End of Intervention (EOI) Clinic Visit**	**Early Termination Clinic Visit**	**Follow-up Visit[5]**
PROCEDURE	**Day -28 to -7**	**Day -10 to -7**	**Day 1 to EOI**	**Refer to Appendix**		**EOI + 3 months (± 2 weeks)**
Platform Protocol Eligibility Criteria (Overall)	X		Refer to Appendix-specific study procedures	Refer to Appendix-specific study procedures		
Informed consent	X					
Demographics	X					
Medical History[6]	X					
Concomitant Medications	X	X			**X**	
Randomization		X				
Coordinated Non-pharmacologic Care[7]						
Appendix Eligibility Criteria	X	X				
Nasal Swab for SARS-CoV-2 rapid antigen test		X				
Clinic visits, as per specific study Appendix					X	X
OHQ/OIQ	X	X[8]			X	X

COMPASS-31	X				X	X
Malmo POTS Symptom Score (MAPS)		X			X	X
Vanderbilt Orthostatic Symptoms Score		X			X	X
Active Stand Test		X	Refer to Appendix-specific study procedures	Refer to Appendix-specific study procedures	X	X
6-minute Walk Test		X			X	X
Modified Depaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM)[9] (look back period of 7 days)		X				
DSQ-PEM[9] (look back period "since last visit")		X			X	X
PROMIS-29 + 2 Questionnaire		X			X	X
PASC Symptom Questionnaire		X			X	X
Blood Collection[10]		X			X	X
Stool Collection		X			X	
Skin Biopsy[11]		X			X	
Autonomic function tests at specialized sites		X			X	
Pregnancy Test		X				
Height and weight		X				
Study Drug						

Administration						
Distribution of wearable[12]		X				
Safety Assessment of AEs including SAEs and ESIs		X			X	X
Adherence[13]					X	

Abbreviations: PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ-Orthostatic intolerance questionnaire; AE-Adverse Events; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1]Some of the eligibility baseline assessments (active stand test and labs), can be performed at the Screening visit if the participant comes in-person for Screening.

[2] Screening cannot be on the same day as the Baseline/Randomization visit.

[3] Eligibility assessments (active stand test, OHQ/OIQ, and labs) should be completed prior to randomization.

[4]A detailed schedule of activities for the Study Intervention Period is provided in each Appendix.

[5] Follow-up visit windows for each appendix:

Appendix A: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 336 (Day 322 to Day 350)

Appendix B: The follow-up visit will occur on EOI + 3 months (± 2 weeks), which corresponds to Day 168 (Day 154 to Day 182)

[6] If medical Records are not available, they are not required. History and physical examination could be performed at screening/baseline visit. At every clinic visit after screening visit, changes in medical history including SARS-CoV-2 infection since last visit will be collected.

[7]Only applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only. During this time, a care coordinator will reach out to the participants weekly.

[8] The OHQ/OIQ completed at Baseline visit will be used to determine eligibility.

[9] The DSQ-PEM will be done twice for the baseline visit: once on the day of the visit with a look back period of “past 7 days” and once within one day after the visit with a look back period of “since last study visit (SLV)”. DSQ-PEM SLV will be administered via phone or electronic survey. The DSQ-PEM administered at every timepoint after baseline will be within one day after strenuous in-person study visits and will ask about the period “SLV”.

[10] Blood collection for safety parameters, biomarkers, and biospecimen for biorepository. Blood collection may also be collected as needed based on PI discretion and adverse events.

[11] Skin biopsy is only applicable to Appendix A: IVABRADINE. If the baseline skin biopsy shows evidence of small fiber neuropathy, the biopsy will be repeated at EOI or ET.

[12] It is required to wear the wearable at least 7 days before start of study drug intervention and 7 days before end of intervention visit. All other times, it is strongly encouraged.

[13] Adherence to drug (pill count) will be documented. Participants receiving oral study drugs to be taken at home will receive weekly phone calls for the first 30 days.

Table 2b. Schedule of Study Intervention Procedures for Appendix B: Ivabradine

	Study Intervention Period		
	Start of Study Intervention	**1 Month Clinic Visit**	**End of Intervention (3 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 28 ± 7 days (Day 21 to Day 35)**	**Day 84 – 7 days (Day 77 to Day 84)**
Concomitant Medications		X	X
Study Drug Administration[1]	X	X	
Coordinated Non-pharmacologic Care[2]	X	X	X
Weekly site calls[3]	X	X	
OHQ/OIQ Assessment		X	X
COMPASS-31		X	X
MAPS		X	X
Stool Collection			X
Active Stand Test and Vanderbilt Orthostatic Symptoms Score		X	X
6-minute Walk Test		X	X
DSQ-PEM[4] (look back period “since last visit”)		X	X

	Study Intervention Period		
	Start of Study Intervention	**1 Month Clinic Visit**	**End of Intervention (3 Month Clinic Visit)**
PROCEDURE	**Day 1**	**Day 28 ± 7 days (Day 21 to Day 35)**	**Day 84 – 7 days (Day 77 to Day 84)**
PROMIS-29 + 2 Questionnaire		X	X
Blood Collection[6]			X
PASC Symptom Questionnaire		X	X
Autonomic function tests at specific sites			X
Safety Assessment of AEs, including SAEs and ESIs	X	X	X
Vital signs monitoring[7]		X	
Adherence[5]	X	X	X

Abbreviations: EOI – End of Intervention, QOL – Quality of Life; PASC-Post-acute sequelae of SARS-CoV-2 infection, OHQ– Orthostatic hypotension questionnaire; OIQ- Orthostatic intolerance questionnaire; AE – Adverse Events; SAE – Serious Adverse Events; ESI – Events of Special Interest

[1]Study drug administration will occur every day during the intervention period

[2] Only applicable for those who are randomized to coordinated non-pharmacologic care. Not applicable to those receiving usual care. The coordinated non-pharmacologic care will be for a total duration of 3 months only.

[3]Participants will receive weekly phone calls for the first 30 days of the study intervention period

[4] DSQ-PEM questionnaire will be via phone or electronic survey within one day after strenuous in-person study visits.

[5] Adherence to drug (pill count) will be documented.

[6] Blood collection may be collected at any time as needed based on PI discretion and adverse events.

[7] HR will be captured at the 1-month clinic visit to determine dosing.

4.2 Efficacy Assessments

4.2.1 Primary Endpoint: Orthostatic Hypotension Questionnaire (OHQ)

The Orthostatic Hypotension Questionnaire (OHQ) is a PRO that measures orthostatic intolerance, which has been the primary presentation of patients with PASC-related autonomic dysfunction. This measure

includes the 6-item symptom assessment (OHSA) and the 4-item Daily Activity Scale (OHDAS). Each item in OHSA and OHDAS is scored from 0 (none/no interference) to 10 (worst possible/complete interference), describing the preceding week. OHDAS items also have a score of 11, representing "Cannot do for other reasons". Prior data in patients with PASC suggest that this measure well-discriminates those with symptoms compared to healthy controls. Measurements from the first 50 subjects, combining IVIG and Ivabradine appendices, will be used to assess test-retest reliability (1).

OHSA and OHDAS are calculated by averaging across items within OHSA and OHDAS, respectively. Operationally, baseline subscores will be averaged over non-zero/non-eleven items to yield a composite score (1). Follow-up subscores will be averaged over items that were non-zero and non-eleven at baseline and follow-up visits. The endpoint is the change in OHQ composite score from baseline to EOI.

All sensitivity and subgroup analyses related to the primary endpoint will depend on funding availability.

4.2.2 Secondary Endpoint: Compass-31

The COMPASS-31 is a patient-reported instrument that measures autonomic symptoms across multiple domains commonly seen in patients with PASC.

COMPASS-31 encompasses six domains, including orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor. Each domain has a set of questions that are scored on a five-point scale, and the final score is obtained by summing the weighted scores of all domains. The total score ranges from 0 to 100, with higher scores indicating a higher severity of autonomic symptoms.

COMPASS-31 composite score (3) is the sum of all 6 domain scores:

1. Orthostatic Intolerance-score: Sum of items 1-4 multiplied by 4
2. Vasomotor-score: Sum of items 5-7 multiplied by 0.8333
3. Secretomotor-score: Sum of items 8-11 multiplied by 2.1428571
4. Gastrointestinal-score: Sum of items 12-23 multiplied by 0.8928571
5. Bladder-score: Sum of items 24-26 multiplied by 1.111
6. Pupillomotor-score: Sum of items 27-31 multiplied by 0.333

The endpoint is the change of the composite score of COMPASS-31 from baseline to EOI.

4.2.3 Secondary Endpoint: Malmo Pots Symptom Score (MAPS)

The MAPS assesses symptom burden in POTS. It is a self-rating, 12-item score (0-10 per item, total range 0-120) based on patients' own perception of symptoms through visual analogue scale assessment. The MAPS composite score (4) is the sum of all 12-items scores.

The endpoint is the change in the Malmo Potts Symptom score from baseline to EOI.

4.2.4 Secondary Endpoint: Active Stand Test – Heart Rate Change

The active stand test is a well-characterized performance measure of short-term neurologic and cardiovascular function that requires limited resources and aids in diagnosis of patient symptoms of orthostatic intolerance.

It is important to standardize and measure change from supine to standing and not from sitting with legs down to standing. Participants will remain supine for 10 minutes, and data will be acquired at 5 and 10 minutes. Standing test should be performed with HR and BP monitoring at 1, 3, 5 and 10 minutes. In patients with orthostatic intolerance this test induces a BP drop within the first minute that correlates with HR and BP responses to tilt up and can be used as a measure of orthostatic intolerance.

Heart rate change (ΔHR) is measured as the (Highest heart rate standing) minus (Lowest supine heart rate) during active stand test. The endpoint is the change in ΔHR from baseline to EOI.

4.2.5 Secondary Endpoint: Lowest Supine Heart Rate in Active Stand Test

The endpoint is the lowest supine heart rate in active stand test from baseline to EOI.

4.2.6 Secondary Endpoint: 6-Minute Walk Test

Normal walking speed will be measured using a standard 6-minute walk, which is an established measure of overall health.

This endpoint is the change in total distance walked (m) in 6 minutes from baseline to EOI. If the participant is unable to complete the 6-minute walk, the total distance walked will still be counted as the endpoint.

4.2.7 Secondary Endpoint: Promis-29 + 2

Patient-Reported Outcomes Measurement Information System (PROMIS-29+2) Profile: 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. The PROMIS-29+2 consists of 31 items: 4 items per domain for each of 7 domains (physical function, anxiety, depression, fatigue, sleep disturbance, ability to participate in social roles and activities, and pain interference), plus 1 item for pain intensity and 2 items for cognitive function. Pain intensity is assessed on scale ranging from 0 (no pain) to 10 (worst pain imaginable). All other items use Likert-type 5-point scales. Scoring of the PROMIS-29+2 will follow established guidelines (5,6). Domain-specific summary scores will be expressed as T-scores with higher scores representing a worse condition for domains measuring impairment (e.g., anxiety, depression, fatigue, sleep disturbance, and pain interference) and representing a better condition for domains measuring function (e.g., physical function, ability to participate in social roles and activities). T scores are interpreted in relation to a US general reference population with population mean = 50 and SD = 10. An overall summary of health-related quality of life will be derived from the PROMIS-29+2 by calculating the PROMIS Preference Score (PROPr).

4.2.8 Exploratory Endpoints: Autonomic Function Tests At Specific Sites

Additional autonomic function testing will be completed at specific sites that have the capability to perform these tests:

Head-up Tilt (HUT) Test - This test stimulates the autonomic nervous system with orthostatic stress by passive movement from a supine position to an upright tilt in a controlled laboratory setting. Tilt test provides both sympathetic and parasympathetic assessments. The variables measured will be continuous BP, HR, and end tidal CO2. The technique used for this assessment will be 10 minutes of

baseline and 10 minutes 70 degree head-up tilt (HUT). The outcome will be all variables at baseline and at every 1 minute of the tilt (total 10 measurements during the tilt).

- Heart Rate (HR)

The baseline measure will use the 10-minute lying down HR, and the endpoint is the difference between each time point HR measurement (1 through 10) at EOI and the baseline measurement. The endpoint will be analyzed both as continuous value and a categorical value (normal/abnormal). A difference in absolute HR equal to or greater than 30 will be considered abnormal.

- Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)

The baseline measure will use the 10-minute lying down SBP and DBP, and the endpoint is the difference between each time point SBP and DBP measurement (at 1, 3, 5, and 10 minute) at EOI and the baseline measurement. The endpoint will be analyzed both as continuous value and a categorical value (normal/abnormal). A difference in absolute SBP equal to or greater than 20 will be considered abnormal. A difference in absolute DBP equal to or greater than 10 will be considered abnormal.

- End-tidal CO2

The baseline measure will use the 10-minute lying down End-tidal CO2, and the endpoint is the difference between each time point CO2 measurement (1 through 10) at EOI and the baseline measurement. The endpoint will be analyzed both as continuous value and a categorical value (normal/abnormal). A difference in absolute CO2 less than 30 will be considered abnormal.

Transcranial doppler (TCD), if available – The test measures the velocity of blood flow (CBFv Systolic and CBFv Diastolic) through the brain's blood vessels by measuring the echoes of ultrasound waves. The baseline measure will use the 10-minute lying down value, and the endpoint is the difference between each time point cerebral blood flow velocity (1 through 10) at EOI and the baseline measurement. The endpoint will be analyzed both as continuous value and a categorical value (normal/abnormal). The low limit for women/men are 82.2–0.45 (cm/s)*age (years)/72.09–0.38 (cm/s)*age (years). The normal decline in orthostatic CBFv from baseline is equal or less than 10% (first minute), 11% (fifth minute), and 15% (10th minute) of the tilt test [6].

Valsalva maneuver – This test evaluates sympathetic adrenergic functions using BP responses and cardiovagal (parasympathetic) functions using the HR responses. Valsalva maneuver consists of forced expiration against the resistance with the expiratory pressure during strain at 40 mm Hg for 15 seconds. The measured variables will be continuous BP and HR. There are four endpoints:

- the change in mean blood pressure (2/3 DBP + 1/3 SBP) phase II late from baseline to EOI;
- the change in mean blood pressure (2/3 DBP + 1/3 SBP) over phase IV minus the start blood pressure systolic from baseline to EOI;
- the change in the ratio between maximum and minimum HR over phase IV from baseline to EOI for sinus arrhythmia;
- the change in time to return to baseline SBP in seconds from baseline to EOI.

Deep breathing test – The test evaluates changes in the instant HR that is provoked by “deep” breathing. The test is performed in supine position. The measured outcomes will be the ratio of the HR during inspiration and expiration as well as the calculated respiratory sinus arrhythmia (RSA). RSA

amplitude is defined as the difference between the end of expiration and end of inspiration in HR. Typically, 6 respiratory cycles are identified, and the respective RSA amplitudes are averaged. The endpoint is the change in the average of difference between maximum HR and minimum HR over 6 respiratory cycles from baseline to EOI.

4.2.9 Exploratory Endpoint: Vanderbilt Orthostatic Symptoms Score (VOSS)

The VOSS consists of 9 orthostatic symptoms rated on a scale of 0 (no symptom) to 10 (worst the participant has experienced) at the end of each HUT and after the active stand test. These symptoms are mental clouding, blurred vision, shortness of breath, rapid heartbeat, tremulousness, chest discomfort, headache, lightheadedness, and nausea.

The endpoint is the change in the VOSS score (the sum of all 9 symptom scores) from baseline to EOI. The maximum score a participant can receive is 90 arbitrary units (AU), and a higher score is indicative of a greater orthostatic symptom burden. The VOSS score is missing if any of the 9 symptom score is missing.

4.2.10 Exploratory Endpoint: PASC Symptom Questionnaire

Participants will be asked to complete a questionnaire that asks about the presence of PASC symptoms. This questionnaire includes additional PASC symptoms that are not directly related to autonomic dysfunction. This questionnaire assesses 25 different PASC symptoms for both frequency and severity and 8 additional questions on a 5-point scale, for a total of 58 variables. Frequency ranges from “Never” to “Almost Constantly”, and Severity ranges from “None” to “Very Severe.” This questionnaire includes additional PASC symptoms that are not directly related to autonomic dysfunction. Two questions, patient global impression of severity (PGI-S) and change (PGI-C), are included in this questionnaire

4.2.11 Exploratory Endpoints: Active Stand Test – Supine Blood Pressure At the Timepoint Lowest Supine Heart Rate Measured

The exploratory active stand test endpoints are as follows:

- The change in ΔSBP = SBP at the timepoint with the lowest supine heart rate at active stand test from baseline to EOI.
- The change in ΔDBP = DBP at the timepoint with the lowest supine heart rate at active stand test from baseline to EOI.

4.2.12 Active Stand Test – Blood Pressure Change

The exploratory active stand test endpoints are as follows:

- The change in ΔSBP = (Highest SBP standing) minus (Lowest supine SBP) from baseline to EOI.
- The change in ΔDBP = (Highest DBP standing) minus (Lowest supine DBP) from baseline to EOI.

4.2.13 Exploratory Endpoints: Tracked Accelerometry Measurements

Wearable device will be distributed at baseline to measure accelerometry (step count and heart rate). It is required to wear the wearable 7 days before start of study drug intervention and 7 days before end of intervention visit. All other times, it is strongly encouraged.

The endpoints are the change in average daily total step count and average heart rate over past 7 days from baseline and end of intervention.

4.2.14 Exploratory Endpoints: Biomarkers

Blood will be collected from participants and sent to a central lab for biomarkers assessment. The biomarkers assessed will include inflammatory cytokines, skin biopsies, and other viral biomarkers, immune biomarkers, Endothelial function biomarkers, and biomarkers for autonomic and cognitive function. The endpoints are the change in the biomarkers from baseline to EOI.

- Viral biomarkers
- Autonomic function biomarkers
- Immune biomarkers
- Inflammatory biomarkers
- Endothelial function biomarkers
- Cognitive function (CCL11)

4.2.15 Safety Endpoints: Modified Depaul Symptom Questionnaire-Post Exertional Malaise.

The Modified Depaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM) PRO assessment. Some patients have experienced debilitating PEM that may occur following exertion or on the day after exertion. To assess PEM, the Depaul Symptom Questionnaire 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 7-day look back period at baseline, and at all other timepoints, the look back period will be since the last study visit.

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 if the participant reports having at least one moderate (rated severity ≥ 2) PEM symptom for a frequency rated ≥ 2.

The DSQ-PEM questionnaire will be via phone or electronic survey within one day after strenuous in-person study visits. This evaluation will allow us to assess the effect of study procedures on exacerbation or development of PEM in a population who may be particularly vulnerable to this condition. 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. An analogous continuous measure will be obtained by counting the number of symptoms for which PEM criteria are met, i.e., the number of symptoms with at least moderate severity occurring with at least 50% frequency.

The PEM will be summarized by treatment group as a continuous variable in the ITT population at baseline and end of intervention.

4.2.16 Quantitative autonomic symptoms score (QASAT)

QASAT (quantitative scale for grading of cardiovascular reflex tests, transcranial Doppler, sudomotor testing, and small fiber densities from skin biopsies) is an objective instrument for grading dysautonomia, related small fiber neuropathy and cerebral blood flow. QASAT uses established autonomic tests (deep breathing, Valsalva maneuver, tilt test, sudomotor test) and skin biopsies for assessment of small fibers [7]. A modified version of QASAT as it contains a subset of items in QASAT. The following items to be included are:

- Heart rate variability, uses deep breathing test:

Results	Grading	Value	Definition
Normal		0	100%=normal limit
Abnormal	Mild	1	<100% and ≥50%
	Moderate	2	<50% and≥ 35%
	Severe	3	<35%

- Valsalva maneuver, blood pressure:

Results	Grading	Value	Definition
	Normal	0	End of phase 2 returns to or exceeds the baseline
	Abnormal Mild	1	≥90% and<100% of baseline
	Moderate	2	<90% and≥60% of baseline
	Severe	3	<60% of baseline

- Orthostatic Hypotension:

Normal		0	At each measurement, assign the value 0 for normal results and
Abnormal	Mild	1,2	and 1 for abnormal results. Baseline is defined as he BP in
	Moderate	3,4,5,6	supine position just before orthostasis. BP should be obtained at
	Severe	>6	baseline and every minute of orthostasis up 10 min for orthostatic testing. For orthostasis longer than 10 min, additional measurements should be obtained every 5 min.

- Orthostatic cerebral hypoperfusion:

Results	Grading	Value	Definition
Normal		0	At each measurement, assign the value 0 for normal results
Abnormal	Mild	1	and 1 for abnormal results. Measurements should be obtained
	Moderate	3,4,5,6	at baseline and every minute of up to 10 min of the orthostatic
	Severe	>6	test. For orthostasis longer than 10 min, additional measures should be obtained every5 min. Total score obtained by sum of all scores.

- Orthostatic hypocapnia:

Results	Grading	Value	Definition
Normal		0	At each measurement, assign the value 0 for normal and 1 for Abnormal results. Abnormal response during the orthostasis is defined as an orthostatic drop of end tidal CO2 > 5 mmHg with final end tidal CO2 > 30 mmHg (relative hypocapnia) or < 30 mmHg (absolute hypocapnia). Measurements should be obtained at baseline, and every minute of orthostasis up 10 min of the orthostasis. For orthostasis longer than 10 min, additional measurements should be obtained every 5 min. Total score is obtained by sum of all scores.
Abnormal	Mild	1,2	
	Moderate	3,4	
	Severe	>4	

- Epidermal nerve fiber density:

Results	Grading	Value	Definition
Normal		0	100% = normal limit
Abnormal	Mild	1	1 point: ≥ 85% and < 100%
	Moderate	2,3,4	2 points: ≥ 65% and < 85%
	Severe	> 4	3 points: ≥ 50% and < 65%
			4 points: < 50%
			Measurements are done at each biopsy sites. Total score is obtained by sum of all scores. At least 1 biopsy should be done at the distal leg (10 cm above the lateral malleolus), with a second site at the thigh (20 cm distal to the iliac spine)

- Sweat gland nerve fiber density:

Results	Grading	Value	Definition
Normal		0	100% = normal limit
Abnormal	Mild	1	1 point: ≥ 85% and < 100%
	Moderate	2,3,4	2 points: ≥ 65% and < 85%
	Severe	> 4	3 points: ≥ 50% and < 65%
			4 points: < 50%
			Measurements are done at each biopsy sites. Total score is obtained by sum of all scores. At least 1 biopsy should be done at the distal leg (10 cm above the lateral malleolus), with a second site at the thigh (20 cm distal to the iliac spine)

The modified QASAT will be summarized by treatment group as a continuous variable in the ITT population at baseline and end of intervention if at least 50 patients having non-missing QASAT score. Otherwise, only the sub-category scores will be presented.

4.2.17 Biorepository for Future Research

The RECOVER Biorepository is designed to collect and store blood plasma, serum samples, and stool samples for future research related to the various studies of the RECOVER Program. Such research might include developing diagnostic and/or prognostic tests, improving our understanding of the underlying pathophysiology of PASC, and developing new therapeutic targets. 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 DCRI which serves as the CT - DCC for all RECOVER clinical trials.

The existence of the biorepository will provide the opportunity to devise new hypotheses, since blood collection techniques are standardized across all approved protocols thereby allowing cross-protocol sample comparisons if scientifically justified.

Within this framework, the design of the biorepository is to collect additional blood and prepare EDTA plasma and serum, to be stored in the biorepository for future, as yet unspecified, analyses and studies. A serologic test of COVID-19 infection will be done as part of the biorepository for all participants. Approximately eighty (80) mL of blood will be collected to prepare the aliquots for storage at -80°C. Specifically, two 1.0 ml aliquots of EDTA plasma and two 1.0 ml aliquots of serum will be prepared. These samples will be stored at the Biorepository in a lab for up to 7 years.

5 GENERAL ANALYSIS CONSIDERATIONS

5.1 Data Sources

At each study site, data will be entered on the electronic Case Report Forms (eCRFs) stored in Medidata Rave. Fitbit data will be received from Care Evolution. Randomization date will be received from ALMAC. Biomarker data will be collected by NIH-selected laboratories and will be integrated with the clinical database. Prior to database lock, programmed computer edit checks will be run against the database to identify discrepancies and verify reasonableness of the data. Queries to resolve discrepancies will be generated and resolved by the sites. As needed, DCRI Statistics will be able to directly download the eCRF database from Rave using SAS on Demand. Analysis datasets will be created by DCRI Statistics for production of final tables, figures, and listings. All programs written to create analysis datasets and perform analyses will be validated according to SOPs established by the DCRI Statistical Programming group.

5.2 Software

The statistical analyses described in this SAP, as well as production of tables, listings, and figures will be performed using SAS®, version 9.4 or higher (SAS Institute, Cary, NC). Additional statistical software may be used as needed.

5.3 Verification of Results

All tables, listings, and graphs will be verified and reviewed before considered final. The verification process will ensure that the numbers are produced by a statistically valid method and that the execution of the computations is correct. Qualified statisticians or statistical programmers employed by the DCRI who have not been previously involved in the production of the original programming will perform the verification procedures. Methods of verification include independent programming, prior to issuance of the draft statistical report, of all analysis datasets and comparison to data listings. Tables, listings, and figures will be reviewed for accuracy, consistency with this analysis plan, consistency within tables/listings/figures, and consistency with corresponding output. Once verification is complete, all

documentation of the verification process will be filed as required by the Statistical Standard Operations Procedures of the DCRI.

5.4 Populations for Analyses

All primary efficacy analyses will be based on an intention-to-treat (ITT) population, consisting of all randomized participants, analyzed according to their randomly assigned treatment group, regardless of adherence or discontinuation. The population for safety analyses will consist of all randomized participants who receive at least part of any randomized study treatment (intervention or control).

Although this SAP pertains primarily to Appendix B, two sets of pre-specified analyses will use data pooled across Appendices A and B:

1. the coordinated non-pharmacologic care versus usual non-pharmacologic care efficacy comparison; and
2. estimation of baseline OHQ test-retest reliability.

These analyses are defined at the platform level and apply consistently across both appendices.

All other analyses described in this SAP will use Appendix B participants only. Unless otherwise stated, references to the ITT or safety population in this SAP refer to Appendix B participants only.

5.5 Primary Analysis: Ivabradine Versus Placebo

Objective.

The objective of this analysis is to estimate the effect of randomization to Ivabradine versus placebo on the primary endpoint, the change in OHQ/OIQ composite score from baseline to the end of intervention (EOI) at Month 3. Appendix B uses a 2×2 factorial design in which participants are independently randomized to (1) Ivabradine versus placebo and (2) coordinated versus usual non-pharmacologic care. The present section focuses solely on the pharmacologic comparison of Ivabradine versus placebo.

Estimand.

The primary estimand is the marginal average treatment effect (ATE) of Ivabradine versus placebo. More specifically, it is the average difference in the Month-3 OHQ/OIQ change score that would be observed if all participants in Appendix B were assigned to Ivabradine versus if all were assigned to placebo, while retaining the actual distribution of non-pharmacologic treatment assignments. This marginal estimand remains well defined even if interaction between pharmacologic and non-pharmacologic interventions is present, although its clinical interpretability may be reduced.

Outcome Model.

Estimation will use a g-computation framework based on a linear regression model with adjustment for the following covariates:

- Indicator of assignment to Ivabradine versus placebo
- Indicator of assignment to coordinated versus usual care
- Pharmacologic × non-pharmacologic interaction,
- Baseline OHQ/OIQ composite score (continuous)
- Ivabradine × baseline OHQ interaction

- Coordinated care × baseline OHQ interaction
- Age (continuous)
- Sex (female, male)
- Body mass index (BMI; continuous).

G-Computation Estimation

For each participant, two predicted outcome values will be generated from the fitted regression model:

1. Predicted OHQ change if assigned Ivabradine
2. Predicted OHQ change if assigned placebo

All other covariates (including the non-pharmacologic assignment variable) will remain fixed at their observed values. When generating predicted outcomes under each hypothetical treatment condition, any model term that depends on the pharmacologic treatment assignment (i.e., interaction terms) will be recomputed to correspond to that hypothetical assignment.

The marginal average treatment effect (ATE) of Ivabradine versus placebo will then be calculated as the average within-participant difference between these two predicted outcomes.:

$$\widehat{\Delta} = \frac{1}{n_{ITT}} \sum_{i} \widehat{Y}_i(\text{Ivabradine}) - \widehat{Y}_i(\text{Placebo})$$

where n_{ITT} is the number of participants in the ITT population.

A Wald-type 95% confidence interval (CI) and p-value will be calculated using sandwich (robust) standard errors.

The null hypothesis is

$$H_0: \Delta = 0.$$

Rejection of this null hypothesis will provide evidence that Ivabradine alters the mean OHQ/OIQ change at Month 3.

5.5.1 Considerations for interaction between pharmacologic and non-pharmacologic treatments

The primary outcome model will include an interaction term between pharmacologic and non-pharmacologic treatment assignments. A Wald test of the interaction coefficient will be reported with a nominal two-sided alpha = 0.05. Given the limited sample size and the exploratory nature of this assessment, this test will be interpreted descriptively; it will not alter the primary estimand.

Regardless of the interaction p-value, the primary estimand will be the marginal average treatment effect (ATE) of Ivabradine versus placebo, averaged over the empirical distribution of non-pharmacologic treatment assignments and baseline covariates. If the estimated interaction is large and/or the interaction p-value is small, the following stratified treatment effects will also be presented:

- Ivabradine versus placebo among participants assigned to coordinated care
- Ivabradine versus placebo among participants assigned to usual care.

Each of these will be reported with 95% confidence intervals. These stratified results will be considered exploratory but may inform the design and targeting of future trials.

5.5.2 Sensitivity analysis with an expanded set of covariates

The covariate adjustment strategy in Section 5.5 is consistent with recent FDA guidance and has two main objectives: (1) to increase statistical power and precision by exploiting information contained in baseline covariates, and (2) to reduce bias from potentially informative missing outcome data by accounting for differences in baseline factors among participants with missing versus non-missing outcomes. Out of concern for the ratio of covariates to sample size, we have limited our adjustment to baseline OHQ score and three additional covariates (age, gender, and body mass). However, there are a number of additional covariates that may be relevant that are not among our pre-specified list, including:

- Race (black, white, Asian, other)
- Years of education (continuous)
- US census division (New England, Atlantic, East North Central, West North Central, South Atlantic, East South Central, West South Central, Mountain, Pacific).

Adjustment for these additional covariates may mitigate against chance imbalance among treatment groups. Because of this, for the primary endpoint, we will conduct a sensitivity analysis adjusting for the pre-specified covariates (baseline OHQ, age, gender, and body mass) and covariates from the list above.

5.6 Primary Analysis: Coordinated Care Versus Usual Care

Overview and Rationale

The protocol specifies that the primary comparisons are the main effects of the pharmacologic and non-pharmacologic interventions within each study appendix. Because coordinated care is defined identically in Appendices A and B, the primary analysis of coordinated versus usual care will be implemented by pooling participants across both appendices. Overall and appendix-specific estimates will be presented. This approach preserves the protocol-defined estimand while improving statistical efficiency and allowing a unified estimation procedure.

Estimand

The target estimand is the marginal main effect of coordinated non-pharmacologic care versus usual care on the primary endpoint (change in OHQ/OIQ composite score from baseline to Month 3). This estimand represents the average difference in Month-3 outcome that would be observed if all participants in both appendices were assigned to coordinated care versus if all were assigned to usual care, while retaining the observed distribution of pharmacologic treatment assignments and all other covariates.

Outcome Model

The primary endpoint will be analyzed using a linear regression model that mirrors the specification used for the Ivabradine versus placebo comparison. Specifically, the model will include all covariates and interaction terms described in the Ivabradine section, with the addition of a three-way interaction of

coordinated-care × pharmacologic-treatment × appendix to allow for potential heterogeneity of the coordinated-care effect across appendices and pharmacologic treatment groups.

Estimation via G-Computation

Estimation will use the same g-computation approach described for the Ivabradine versus placebo comparison. For each participant, predicted outcomes will be generated under two hypothetical assignments:

1. coordinated care, and
2. usual care.

Model terms involving the coordinated-care assignment (i.e., interaction terms) will be updated to reflect each hypothetical assignment. All other covariates will remain at their observed values. The marginal main effect will then be estimated as the average within-participant difference between these predicted outcomes.

Assessment of Interaction

An interaction test with 3 degrees of freedom will be conducted to assess whether the effect of coordinated care versus usual care differs across the 4 combinations of appendix and pharmacologic treatment assignment. A nominal two-sided p-value less than 0.05 will be regarded as evidence that the coordinated care treatment effect differs across depending on trial or pharmacologic treatment. If a significant interaction is detected, treatment effect estimates stratified by four combinations of appendix and pharmacologic treatment will be of particular interest. Regardless of statistical significance, analyses stratified by appendix and by the combination of appendix and phamacologic treatment will be reported to facilitate interpretation.

5.7 Secondary Endpoints

Secondary endpoints will be analyzed in parallel with the primary comparisons of:

(1) Ivabradine versus placebo, and
(2) coordinated non-pharmacologic care versus usual non-pharmacologic care.

For the Ivabradine versus placebo comparison, analyses of secondary endpoints will be restricted to Appendix B participants.

For the coordinated non-pharmacologic care versus usual non-pharmacologic care comparison, analyses of secondary endpoints will use data pooled from Appendices A and B.

For each secondary endpoint and treatment contrast, the primary analysis will use a linear regression model of the form:

$$Y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \sum_{k=4}^{K} \beta_k x_{ki} + \varepsilon_i$$

where i indexes study participants, Y_i is the change in the secondary endpoint from baseline to end of intervention, x_{1i} is the randomized treatment indicator for the comparison of interest (Ivabradine versus placebo or coordined care versus usual care), x_{2i} is the baseline value of the secondary endpoint, x_{3i} is the randomized treatment indicator for the other treatment condition (pharmacologic or non-

pharmacologic, as applicable), and $x_{4i}, \ldots, x_{Ki}$) are additional pre-specified covariates consisting of age, sex, BMI. For the coordinated versus usual care comparison, the pre-specified covariates will also include a set of indicators representing the combination of appendix and pharmacologic treatment assignment. The parameters $\beta_1, \ldots, \beta_K$ denote the corresponding regression parameters.

Inferences will be based on β_1, which is interpreted as the average treatment effect of treatment, marginalized over the other treatment assignment and conditional on the covariates. In addition to obtaining the p-value from the hypothesis test for β_1, we will also provide the adjusted point estimate of β_1 and its 95% confidence interval. All inferences will be conducted using robust standard errors to minimize unnecessary assumptions on the error distribution.

5.8 Safety

The analyses are based on all randomized participants who receive at least part of the randomized study intervention or control. Serious adverse events (SAEs) and events of special interest (ESIs) will be analyzed and presented as the proportion of participants who experience each specific serious adverse event, as well as the overall proportion of participants who experienced at least one event by treatment. Additionally, the proportion of participants that experience an AE, SAE, or ESIs that leads to treatment discontinuation will be presented. Incidence of ESIs will be listed.

5.9 Exploratory Endpoints

All analyses of exploratory endpoints will use descriptive statistics. The production of exploratory analyses will depend on funding availability and percentage of data collected to ensure statistical meaningful.

5.10 Missing Data

A particularly strong effort will be made to minimize participant loss to follow-up and ensure the completeness of outcome data.

5.10.1 Primary handling of missing outcome data

The primary analyses will use a g-computation framework that provides efficient and valid estimation under a missing-at-random (MAR) assumption. Participants with missing outcome data will not contribute to parameter estimation but will contribute to the covariate adjustment used to infer treatment effects. In g-computation, predicted outcomes are averaged over the empirical distribution of baseline adjustment variables for all randomized participants, including those with missing outcome data. This approach yields consistent estimation of the marginal average treatment effect provided that outcome data are MAR conditional on the covariates included in the adjustment model.

Under this specification, the g-computation estimator coincides with the maximum-likelihood estimator of the difference in means in the presence of missing outcome data. Consequently, it provides a principled likelihood-based missing data framework with proper propagation of uncertainty through both the outcome model and the averaging step. Empirical (sandwich) variance estimators will ensure appropriate reflection of uncertainty due to both estimation and missingness.

5.10.2 Sensitivity analysis with inverse probability weighting

To assess the robustness of the primary analysis, a sensitivity analysis will be conducted using an augmented inverse-probability-of-missingness weighted (AIPW) approach. As in the primary analysis, the targeted estimand is the marginal treatment effect (difference in means). The AIPW estimator has two models, one for missingness and one for the outcome conditional on covariates. It is doubly robust, i.e., it is consistent if either the missingness model or the outcome model is correct, and, if both are correctly specified, it achieves the semiparametric efficiency bound.

For each treatment group, a logistic regression model will be fit to estimate the probability of the event $I(A = a, R = 1)$, where $A = a$ denotes randomization to treatment group a and $R = 1$ denotes presence of a non-missing primary outcome. Explanatory variables will be identical to the primary efficacy analysis. Letting $\hat{\pi}_a(x) = P(R = 1, A = a|X = x)$, each participant with non-missing outcome in group a will receive a weight of $1/\hat{\pi}_a(x)$.

For predicting outcomes, a linear regression model, identical in structure to that used for the primary efficacy analysis, will be fit. Letting $\hat{m}_a(X) = E[Y|X, A = a]$, the form of the AIPW estimator is

$$\hat{\mu}_a = \frac{1}{n}\sum_i \{\hat{m}_a(X_i) + \frac{1(A_i = a, R_i = 1)}{\hat{\pi}_a(X_i)}[Y_i - \hat{m}_a(X_i)\ ]\}$$

and the estimated marginal average treatment effect is given by $\hat{\Delta} = \hat{\mu}_1 - \hat{\mu}_0$. An empirical (sandwich) standard error will be calculated for $\hat{\Delta}$ and 95% confidence intervals will be reported.

This estimator provides an approximately unbiased assessment of the marginal treatment effect, assuming that missingness is ignorable after conditioning on model covariates and that at least one of the models $\hat{\pi}_a(x)$ and $\hat{m}_a(x)$ is correctly specified. Consistency between the primary g-computation results and this AIPW sensitivity analysis will support the robustness of conclusions regarding the primary endpoint.

5.10.3 Sensitivity analysis with multiple imputation

In addition to weighting, a multiple imputation approach will be used to assess the sensitivity of efficacy analyses to OHQ score missingness. Missing data are assumed to be MAR. For the comparison of Ivabradine and placebo, we can utilize the longitudinal measurements of the OHQ. Specifically, we will use multiple imputation implemented via fully conditional specification(12,13) with predictive mean matching(13–15). Multiple imputation will be conducted separately by randomized treatment arm (Ivabradine versus placebo, marginalizing over the non-pharmacologic intervention; coordinated care versus usual care, marginalizing over the pharmacologic intervention) to avoid imposing cross-arm borrowing of information in the imputation model. Results will be combined across imputations according to Rubin's rules(15).

The fully conditional specification approach, also referred to as sequential multivariate imputation and chained equations, is a strategy for handling non-monotone missing data. Its implementation requires specification of the imputation model. Our imputation models will include the following predictors: pharmacological treatment group (Ivabradine versus placebo), non-pharmacological treatment (coordinated non-pharmacological care versus standard non-pharmacological care), baseline covariates

(age, gender, body mass), OHQ scores Y_j for $j = 0, 3$ months, and the interaction between baseline OHQ score and pharmacological treatment assignment (Ivabradine versus placebo). Including intermediate OHQ values as predictors in the imputations for later timepoints helps to preserve the longitudinal trajectory of each participant.

Imputations will be performed using the fully conditional specification, and each OHQ timepoint with missing data will be imputed from its own univariate conditional distribution. Given that the OHQ score is bounded and may be non-normally distributed, skewed, or exhibit clumping, predictive mean matching will be used. Predictive mean matching preserves the empirical distribution of the OHQ scores, avoids out-of-range imputations, and is robust to model misspecification. Each predictive mean matching step will use k-nearest-neighbor donor selection (k = 5), selecting observed values with predicted means closest to the predicted mean of the missing case.

The following sequence of conditional models will be used:

1. For OHQ at month 1: $Y_1|Y_0, T_{pharma}, T_{nonpharma}, T_{pharama}Y_0, X,$
2. For OHQ at month 3: $Y_3|Y_0, Y_1, T_{pharma}, T_{nonpharma}, T_{pharma}Y_0, X,$ and

where T_{pharma} denotes the indicator for assignment to Ivabradine or placebo, $T_{nonpharma}$ denotes the indicator for assignment to coordinated or usual non-pharmacologic treatment, and X denotes baseline covariates.

A minimum of $m = 50$ imputations will be generated to ensure stable estimates of between-imputation variability. If the number of incomplete cases is greater than 50, then the number of imputations will be set to the number of incomplete cases. Convergence of the fully conditional specification algorithm will be monitored using trace plots of the imputed means, autocorrelation diagnostics, and comparison of observed versus imputed distributions at each time point. If diagnostics indicate poor mixing or convergence issues, the number of iterations per chain or donor pool size may be increased.

Once the m imputed datasets are generated, each dataset will be analyzed using the same g-computation approach as in the primary analysis. The resulting point estimates and variances will be combined across imputations using Rubin's rules to obtain a pooled estimate, standard error, confidence interval, and p-value for the causal estimand. If we let $\hat{\psi}^{(m)}$denote the marginal average treatment effect estimate for the mth imputed dataset and $\widehat{U}^{(m)}$denote the corresponding variance estimate, then the pooled estimate is given by

$$\bar{\psi} = \frac{1}{m} \sum_{j=1:m} \hat{\psi}^{(j)}.$$

The total variance is given by

$$V = \frac{1}{m}\sum_{j=1:m} \widehat{U}^{(j)} + \frac{1}{m-1}\sum_{j=1:m} \left(\hat{\psi}^{(j)} - \bar{\psi}\right)^2.$$

Then, a 95% CI is given by $\bar{\psi} \pm z_{0.975}\sqrt{V}$ where $z_{0.975}$ is the corresponding quantile from the standard normal distribution.

5.11 Planned Subgroup Analysis

The following pre-specified subgroup analyses will be conducted to explore potential heterogeneity of treatment effects on the primary endpoint. These subgroups are based on known or hypothesized clinical significance. These analyses will be considered exploratory. Subgroups will be defined using baseline characteristic collected before randomization, and include:

- Sex (male, female)
- Race (white, nonwhite)
- MAPS (Malmo Pots Symptom) composite score at baseline (< 75, ≥ 75)
- COMPASS-31 composite score at baseline (<50, ≥50)
- VOSS (Vanderbilt Orthostatic Symptoms Score) composite score at baseline (<38, ≥38)
- Active stand test heart rate change at baseline (highest standing minus lowest supine) (<72, ≥72 beats/minute)
- Baseline lowest supine pulse heart rate (<72, ≥72 beats/minute)
- Baseline highest standing pulse heart rate (<108, ≥108 beats/minute)
- Baseline BMI (< 26, ≥ 26 kg/m^2)

<u>Additional pre-specified subgroup for coordinated care versus usual care analysis</u>

- Six minute walk test at baseline (<360 meters, ≥360 meters)

Separate subgroup analyses will be performed for the Ivabradine versus placebo comparison and the coordinated care versus usual care comparison. For each primary comparison and subgroup, the primary outcome model will be augmented by adding a subgroup indicator and its interaction with the randomized treatment indicator for the comparison of interest. Subgroup-specific treatment effects will be estimated using the same g-computation framework as in the primary analysis. For each participant, the fitted regression model will be used to generate predicted outcomes under each treatment condition of interest (e.g., Ivabradine and placebo), holding all other covariates at their observed values. The subgroup-specific marginal average treatment effect will be calculated as the mean within-participant difference between these predicted outcomes within the subgroup. Robust (sandwich) standard errors will be used to construct 95% confidence intervals for each subgroup-specific effect. A Wald test of treatment-by-subgroup interaction will be conducted to assess whether the treatment effect differs across levels of the subgroup variable. Given the number of subgroup variables and the limited sample size, no formal adjustment for multiple comparisons will be made. Interaction p-values will be regarded as descriptive and exploratory rather than definitive tests of effect modification.

Interpretation will focus on the magnitude, precision, and clinical plausibility of patterns in the subgroup-specific estimates rather than on any single statistically significant result.

5.12 Baseline Descriptive Statistics

Baseline characteristics will be summarized by arm for the ITT study population. For continuous measures the median (Q1, Q3) will be summarized. Categorical variables will be described by the proportion in each category (with the corresponding sample size). Baseline characteristics will include age, sex, race, ethnicity, level of education, preferred language (English vs. Spanish), blood pressures (systolic and diastolic), heart rate, height, and medical history.

5.13 Considerations for Multiple Comparisons

This Appendix evaluates two prespecified primary comparisons on the same primary endpoint (change in OHQ/OIQ composite score from baseline to Month 3):

1. Ivabradine versus placebo, and
2. Coordinated non-pharmacologic care versus usual non-pharmacologic care.

Because this is a Phase II, signal-seeking study and the pharmacologic and non-pharmacologic interventions address distinct scientific questions, no formal adjustment will be made to control the family-wise type I error rate across these two comparisons. Each primary comparison will be tested at a nominal two-sided alpha = 0.05, and inferences will be made for each comparison separately.

For secondary endpoints, two-sided p-values and 95% confidence intervals will also be reported without formal adjustment for multiple comparisons. These analyses are considered supportive and exploratory; emphasis will be placed on the magnitude and precision of treatment effect estimates rather than on any specific p-value threshold.

Subgroup and interaction analyses will also be regarded as hypothesis-generating. P-values from these analyses will be described as nominal, with no correction for multiple testing, and will be interpreted cautiously in the context of the overall pattern of results.

5.14 OHQ Test-Retest Reliability

A pooled analyiss of Appendix A + B data will be performed to assess the OHQ composite score's test-retest reliability at baseline. For details, refer to the SAP for Appendix A.

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7. Appendix 1. Roster of Statisticians

Team	Name	Email	Attends open DSMB Meetings	Attends closed DSMB Meetings
Unblinded	Frank Rockhold	frank.rockhold@duke.edu	X	X
	Sophia Waymyers	sophia.waymyers@duke.edu	X	X
Blinded	Nikki Freeman	nikki.freeman@duke.edu	X	
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	Jun Wen	Jun.wen@duke.edu	X	

8. Appendix 2. SAP Revision

Revision Date MMM dd, yyy	Section	Summary of Revision	Reason for Revision