UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF ENSITRELVIR (S-217622) FOR VIRAL PERSISTENCE AND INFLAMMATION IN PEOPLE EXPERIENCING LONG COVID (PREVAIL-LC)

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Clinical Research Protocol

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Sponsor:	University of California, San Francisco
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Principal Investigator:	Name: Timothy J. Henrich, MD, MMSc [redacted]

Approval:

December 5th, 2024

Timothy J. Henrich

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing University of California, San Francisco with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number:

Protocol Title:
PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF ENSITRELVIR (S-217622) FOR VIRAL PERSISTENCE AND INFLAMMATION IN PEOPLE EXPERIENCING LONG COVID (PREVAIL-LC)

Protocol Date: 2024-FEBRUARY-21

December 5th, 2024

Investigator Signature *Date*

Timothy J. Henrich, MD Professor of Medicine

Print Name and Title

Site # 1

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CFR	Code Of Federal Regulations
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRP	C-Reactive Protein
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
ESR	Erythrocyte Sedimentation Rate
FDA	Food And Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act Of 1996
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
LIINC	Long-term Impact of Infection with Novel Coronavirus
mEq	Milliequivalent
mITT	Modified Intention-to-Treat
PI	Principal Investigator
PK	Pharmacokinetic
PRN	Pro Re Nata
SAE	Serious Adverse Experience
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SMC	Safety Monitoring Committee

PROTOCOL SYNOPSIS (TO BE COMPLETED LAST)

TITLE	PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF ENSITRELVIR (S-217622) FOR VIRAL PERSISTENCE AND INFLAMMATION IN PEOPLE EXPERIENCING LONG COVID (PREVAIL-LC)
SPONSOR	University of California, San Francisco
FUNDING ORGANIZATION	University of California, San Francisco
NUMBER OF SITES	1
RATIONALE	There is growing evidence that viral antigen persistence and immune dysregulation are primary drivers of Long COVID (LC) symptoms. Recent studies have demonstrated viral persistence in a subset of individuals with LC, including prolonged gastrointestinal shedding, ongoing Spike and Nucleocapsid antigen in neuronal and astrocytic exosomes, and evidence of persistent viral RNA or proteins in blood and in deep tissues. Furthermore, we and others have recently showed that individuals with LC have elevated levels of inflammation, particularly IL-6, IL-1beta and TNF-alpha, while other groups have implicated excess clotting as a mechanism for end-organ disease. Theoretically, virus persistence may be driving inflammation and clotting, leading to the diffuse tissue damage and the symptoms which we associate with Long COVID. If this model is correct, then blocking virus replication will result in reduced tissue damage, either directly or indirectly, and resolution of symptoms. Recent anecdotal experience with readily available antiviral drugs (*e.g.*, Paxlovid) in people with LC provide support for this concept. The finding that LC is less common in people who were vaccinated prior to infection is also indirect evidence for this model. Ensitrelvir (S-217622), an investigational 3CL protease inhibitor administered daily, has been shown to result in rapid clearance of SARS-CoV-2 during acute infection. The agent was well tolerated with few discontinuations due to adverse events and no serious adverse events.
STUDY DESIGN	Randomized, double-blind, placebo-controlled study. 20 participants will receive 5 days of oral ensitrelvir and 20 participants will receive 5 days of oral placebo. Following study unblinding, participants that received placebo will have the option of a 5 day course of ensitrelvir with repeat safety, clinical and laboratory measures.
PRIMARY OBJECTIVE	To evaluate the safety and tolerability of oral ensitrelvir in individuals who are experiencing Long COVID.

SECONDARY OBJECTIVES	1. Describe the ability of ensitrelvir to affect virologic markers of chronic SARS-CoV-2 infection. 2. Describe the ability of ensitrelvir to improve PASC outcomes. 3. Describe the ability of ensitrelvir to reduce post-COVID inflammation.
NUMBER OF SUBJECTS	40
SUBJECT SELECTION CRITERIA	**INCLUSION CRITERIA** 1. Age ≥18 and <70 years at Screening. 2. Enrolled or willing to enroll and complete at least 1 visit in the UCSF Long-term Impact of Infection with Novel Coronavirus (LIINC) study. Any adult who has been infected with SARS-CoV-2 or has ever received or is eligible to receive a SARS-CoV-2 vaccination, and who is able to provide written informed consent, is eligible to participate in LIINC. 3. History of at least one SARS-CoV-2 infection, defined as report of a positive nucleic acid amplification test (NAAT) and/or a positive SARS-CoV-2 antigen rapid diagnostic test (RDT). Written proof of the test will be requested but is not required as long as the participant attests to the positive test. 4. Clinical evidence of Long COVID, as confirmed by the investigator's assessment. a. At least two moderate symptoms or at least one severe symptom as assessed by the study team (see list) that are new or worsened since the time of a SARS-CoV-2 infection, not known to be attributable to another cause upon assessment by the PI. Symptoms from those listed here must be present: systemic symptoms (e.g., fatigue, chills, post-exertional malaise), neurocognitive symptoms (e.g., trouble with memory/concentration ("brain fog"), headache, dysautonomia/postural orthostatic tachycardia syndrome, dizziness, unsteadiness, neuropathy, sleep disturbance), cardiopulmonary symptoms (e.g., chest pain, palpitations, shortness of breath, cough, fainting spells), musculoskeletal symptoms (e.g., muscle aches, joint pain), gastrointestinal symptoms (e.g., nausea, diarrhea). Although other symptoms (e.g., skin rash, hair loss, mental health symptoms, trouble with smell/taste, genitourinary symptoms) will be recorded and tracked, at least two core symptoms listed above must be present. Note: the two symptoms can be from within the same category (for example, brain fog and headache). AND

b. Symptoms must have been present for at least 60 days prior to screening. Symptoms that wax and wane must have been initially present at least 60 days prior to screening.

AND

c. Symptoms must be reported to be at least somewhat bothersome and to have an impact on quality of life and/or everyday functioning.

AND

d. At least 90 days have elapsed since the most recent suspected or confirmed SARS-CoV-2 infection and the time of screening. Note: suspected infections will be determined based upon assessment by the study investigators.

5. Not currently hospitalized.
6. Body mass index (BMI) 18 to 50 kilograms/meter squared (kg/m2), inclusive, at the time of screening.
7. In otherwise stable health, as assessed by the investigator within 28 days prior to Screening, based on medical history, physical assessment, laboratory findings, and vital signs.
8. For Male participants,

   Participants with partners that are women of childbearing potential (WOCBP) are strongly advised to inform their partners and must agree to use effective contraception from study entry (defined as D0) through 14 days after the last dose of study intervention. Effective methods of contraception are described in Appendix 2. Participants with pregnant partners must agree to use condoms during vaginal intercourse from study entry (defined as D0) through 14 days after the last dose of study intervention administration. Participants assigned male sex at birth must agree to refrain from sperm donation from study entry through 14 days after the last dose of study intervention administration.
9. For Female participants,

   A female participant is eligible to participate if she is not pregnant or breastfeeding, and the following conditions applies:

   - Is not a WOCBP

   OR

   - All of the following apply:

1. Is a WOCBP and using a contraceptive method that is effective as described in Appendix 2 from –21 days from study entry (defined as D0), during the study intervention period, and for at least 14 days after the last study intervention administration. She

must also agree not to donate her eggs (ova, oocytes) for the purpose of reproduction from study entry (defined as D0), during the study intervention period, and for at least 14 days after the last study intervention administration. (Of note, participants taking hormonal contraception will be informed that there may be a possibility of decreased efficacy or contraceptive failure while on study drug.)

- ✓ A WOCBP must have a negative urine pregnancy test within 24 hours before the first dose of study intervention. If a urine pregnancy test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test must be negative.

10. Willingness and ability to comply with the study protocol. This includes reliable transportation and sufficient time to attend all visits.
11. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

EXCLUSION CRITERIA

1. Previously received COVID-19 convalescent plasma treatment or a SARS-CoV-2-specific mAb within 60 days prior to planned Day 0 or plan to receive such treatment before exiting the study.
2. Previously received a SARS-CoV-2 antiviral within 60 days prior to planned Day 0 or plan to receive such treatment before exiting the study.
3. Plans to receive any investigational or approved vaccine or booster for SARS-CoV-2 within 60 days prior to planned Day 0 or before Day 30 following planned Day 0.
4. Active cardiovascular disease, defined as known:
    a. Myocardial infarction within 90 days of screening

       OR

    b. Coronary artery bypass procedure within 90 days of screening

       OR

    c. Current heart failure with reduced ejection fraction (<45%)

       OR

    d. Current pulmonary arterial hypertension.
5. Known stroke within 3 months prior to planned Day 0.
6. Known active bacterial, fungal, viral, or other infection besides SARS-CoV-2 requiring treatment within the 28 days prior to planned Day 0 and meeting criteria for systemic involvement upon review by the PI. Note: Mild or limited infections such as

	uncomplicated urinary tract or yeast infections, sexually transmitted infections, and mild dermatophyte infections may be reviewed with the study PI and/or Safety Monitoring Committee chair, but are not exclusionary. 7. Major surgery within 6 months prior to planned Day 0 or planned major surgery during the first 60 days following planned Day 0. 8. History of unplanned hospitalization for >24 hours within 28 days prior to Screening. 9. Active Hepatitis B (Hep B) infection (defined as Hep B surface antigen (sAg) positive). Note: A known positive Hep B core antibody (cAb) in the absence of positive sAg is not considered exclusionary. 10. Active Hepatitis C (Hep C) infection (defined as Hep C Ab positive or indeterminate with detectable Hep C RNA). Note: Those with cured Hep C (Ab positive or indeterminate but negative Hep C RNA) will remain eligible. 11. Laboratory abnormalities at Screening, specifically including: a. ANC <1500 per mm^3 b. Platelet count <100,000 per mm^3 c. AST/ALT > 1.5x ULN d. CrCl < 30 mL/min e. Hemoglobin <9 g/dL 12. Known HIV infection. 13. End stage kidney disease requiring dialysis. 14. Severe hepatic impairment (Child-Pugh Class C). 15. Moderate or severe immunocompromise, according to the current NIH COVID-19 Treatment Guidelines as of March 6, 2023. The detailed list is in Appendix 2, and includes the following: (a) receiving active treatment for solid tumor or hematologic malignancy, including use of systemic chemotherapy for treatment of cancer within the year prior to screening, (b) prior solid-organ transplant with active immunosuppressive therapy, (c) CAR-T cell therapy or hematopoietic cell transplant, on immunosuppressive therapy or transplant within the prior 2 years, (d) primary immunodeficiency syndromes, advanced or untreated HIV infection (any HIV infection is exclusionary), (f) on active high-dose corticosteroids (i.e., >= 20mg prednisone or equivalent daily per day for >= 2 weeks) at the time of screening. 16. Known prior diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), preceding and not related to SARS-CoV-2 infection and not worsened since SARS-CoV-2 infection. 17. Known prior diagnosis of dysautonomia, preceding and not related to SARS-CoV-2 infection and not worsened since SARS-CoV-2 infection.

	18. Pregnant, breastfeeding, or unwilling to practice birth control abide by the contraception requirements outlined in the inclusion criteria. 19. Participation in a clinical trial with receipt of an investigational product within 28 days or 5 half-lives (whichever is longer) prior to planned Day 0. The following exceptions will apply: a. If the investigational product is a SARS-CoV-2-specific monoclonal antibody, convalescent plasma, or equivalent (e.g., IVIG), refer to Criterion #1 (60 days must have elapsed prior to planned D0). b. If the investigational product is a PET imaging tracer that is not expected to have significant biological activity, has a biological half-life less than 24 hours and radiological half-life less than 6 hours; more than 14 days must have elapsed between receipt of the tracer and planned Day 0. 20. Current alcohol or illicit drug use as determined by the investigator to preclude participation. 21. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data. 22. Study site personnel directly affiliated with the study or family of directly involved personnel. 23. Known allergy to any components used in the formulation of the intervention. 24. Current use of or anticipated need for any medications prohibited with study intervention, as described in Appendix 3. 25. Participants who have used any of the following drugs within 14 days prior to enrollment: ✓ Strong cytochrome P450 (CYP) 3A inducer 2. Products containing St. John's wort
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Ensitrelvir Fumaric Acid (S-217622) given orally 375 mg on day 1 followed by 125 mg daily for 4 additional days
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Placebo, given orally daily for 5 days

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Screening: up to 28 days Treatment: 5 days Follow-up: 60 days For optional cross-over from placebo to study drug arm following main study unblinding: Treatment: 5 days Follow-up: 60 days
CONCOMMITANT MEDICATIONS	All concomitant medication and concurrent therapies taken within 28 days prior to Screening will be documented. Dose, route, unit frequency of administration, and indication for administration and actual or estimated dates of medication will be captured. Details regarding all SARS-CoV-2 vaccinations and/or boosters, as well as COVID-specific therapies, will be collected.
EFFICACY EVALUATIONS	• The change in patient-reported outcomes (PROs) PROMIS-29 score from Baseline to D30.
PRIMARY ENDPOINT	• Safety and tolerability.
SECONDARY ENDPOINTS	• Change in other assessments (EuroQoL Quality of Life, neurocognitive assessment, DASI, 6MWT performance) between Baseline and at D15, D30, and D60.
OTHER EVALUATIONS	• Percentage of participants with no detection of SARS-CoV-2 plasma remnants (*i.e.,* viral detection by reverse transcriptase-polymerase chain reaction ((RT-PCR) and Spike protein fragments) compared to baseline and at D15 and D30 post administration. • Proportion with reduction in inflammatory markers (e.g., interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha) at Baseline and at D15 and D30.
SAFETY EVALUATIONS	Number of participants with adverse events (AE)s (Treatment Emergent Adverse Events (TEAE)s, Serious Adverse Events (SAE)s, and Adverse Events of Special Interest (AESI)). The primary outcome will be the number of individuals in each group experiencing a severe (Grade 3 or greater) AE thought to be possibly, probably, or definitely related to study treatment throughout the study duration.
PLANNED INTERIM ANALYSES	Approximately 3 months after enrollment of the first participant and then every 3 months thereafter, the Independent Medical Monitor (IMM) will review accrual (including screening and enrollment), AE summaries, including all reported Grade ≥3 AEs, retention of participants including off-study rates. Serious adverse events will be monitored by the IMM on an ongoing basis throughout the study.

STATISTICS **Primary Analysis Plan**	Eligible patients who are randomized into the study and receive at least one dose of the study drug during initial blinded, placebo-controlled phase will comprise the modified intent to treat (mITT) population and will be the primary analysis population for all analyses. Exploratory analyses will include mITT population with addition of participants original assigned to placebo who opt to undergo ensitrelvir therapy following unblinding in the optional cross-over stage. Safety and tolerability data will be summarized by treatment group. Adverse event rates will be coded by body system. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. Adverse events between groups will be summarized by proportions with associated 95% Clopper-Pearson confidence intervals and will be compared using a two-sided 0.05 level Fisher exact test.
Rationale for Number of Subjects	This is an exploratory study, and the primary results will be descriptive. We will use ensitrelvir as a probe to better understand LC biology and determine whether a signal is present to warrant further study. For PROMIS-29, assuming a standard deviation (SD) of the within-person difference of 10, then the planned sample size will have 80% power to detect a difference of 11.2 on a two-sided 0.05 level test. For biomarkers, such as IL-6, we will be able to detect a difference from a mean value of 2.5 pg/mL to 1.1 times the within-person standard deviation with 80% power.

1 BACKGROUND

Coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Coronavirus type 2 (SARS-CoV-2), a ribonucleic acid (RNA) virus which emerged in Wuhan, China, in December 2019 and spread across the world at an unprecedented pace through human-to-human transmission. The spike protein of the 2019 novel coronavirus is a surface protein which binds to angiotensin-converting enzyme-2 (ACE-2) on human cells. The S1 subunit catalyzes attachment to ACE-2 and the S2 subunit allows fusion with cell membranes and subsequent entry into the cell. As a result, the spike protein is a relevant target for drug development. Moreover, antibodies (Abs) against the spike protein are able to neutralize the virus, prevent infection, and reduce the severity of disease.

The COVID-19 pandemic has resulted in a growing population of individuals recovering from SARS-CoV-2 infection. Approximately one in five American adults who have had COVID-19 still have symptoms of Long COVID, a type of post-acute sequelae of SARS-CoV-2 infection (PASC).[1] Some aspects of this recovery may be unique to COVID-19, but many appear to be similar to recovery from other viral illnesses, critical illness, and/or sepsis.[2] LC is comprised of a broad range of symptoms that develop during or after COVID-19, continue for ≥2 months (i.e., three months from the onset of illness), have an impact on the patient's life, and are not explained by an alternative diagnosis. Consensus around the clinical definition of LC has progressed significantly, and as of October 1, 2021, there has been an International Classification of Diseases, Tenth Revision, Clinical Modification, (ICD-10), for unspecified post-COVID conditions (U09.9). The World Health Organization (WHO) has also created a global COVID-19 clinical platform CRF for clinicians and patients to collect and report information.[3] The United States (US) Department of Health and Human Services (HHS) and the Department of Justice (DoJ) released a guidance statement on LC as a disability under the Americans with Disabilities Act, the Rehabilitation Act of 1973, and the Patient Protection and Affordable Care Act.

Persistent viral infection with viral reservoirs after the initial acute illness is one potential pathogenic mechanism for Long COVID.[4] Theoretically, virus persistence may be driving inflammation and clotting, leading to the diffuse tissue damage and the symptoms which we associate with Long COVID. If this model is correct, then blocking virus replication will result in reduced tissue damage, either directly or indirectly, and resolution of symptoms. Recent anecdotal experience with readily available antiviral drugs (*e.g.*, Paxlovid) in people with LC provide support for this concept.[5] The finding that LC is less common in people who were vaccinated prior to infection is also indirect evidence for this model. Therefore, this trial will study the safety and efficacy of S-217622, an investigational 3CL protease inhibitor to treat individuals with Long COVID in an adult population.

1.1 Overview of Pre-Clinical & Clinical Studies

Ensitrelvir fumaric acid (S-217622; hereafter, ensitrelvir) is a novel oral SARS-CoV-2 3C-like protease inhibitor which has shown to have antiviral efficacy against many SARS-CoV-2 variants, including Omicron subvariants.[6-9] A phase 1 study demonstrated that one-daily oral doing was well tolerated with favorable pharmacokinetics (**c**). In phase 2a study, ensitrelvir led to a reduction in SARS-CoV-2 RNA levels and viral titers compared to placebo in acutely infected individuals.[10] Subsequent data from the phase 2b part of a phase 2/3 study in 341 participants the change from baseline in SARS-CoV-2 titers on day 4 as significantly greater in both of the oral Ensitrelvir dosing arms [125 mg (375 mg on day 1) or 250 mg (750 mg on day 1) once daily for 5 days] compared with placebo (-0.41 log10 TCID50) and a faster time-weighted average change from baseline to 120 hours in some acute and respiratory symptoms.[11] Adverse events were generally mild and included headache, diarrhea and a transient increase in HDL (which was more pronounced in the higher dosage group)[11]. In a post-hoc analysis, post hoc analysis, compared with placebo, ensitrelvir demonstrated a reduced time to resolution of 5 symptoms in patients with mild-to-moderate COVID-19.[8]

2 STUDY RATIONALE

Early in the pandemic, the common assumption was that SARS-CoV-2 would prove to be a transient infection, as is the case with coronaviruses in general. This assumption was challenged by early reports that viral nucleic acid and proteins could be detected in the gut mucosa months after infection.[12] Case reports emerged indicating that immunocompromised individuals including those with advanced malignancy, Human immunodeficiency virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), or on immunosuppression for autoimmune conditions, can harbor active replicating virus for many months.[13-16] More recently, similar observations have been made in immunocompetent people.[16-21] Autopsy studies of people post-COVID dying from related or unrelated reasons also began to report presence of viral nucleic acid or protein in various tissues months after the infection was apparently cleared.[20] Another provocative study demonstrated SARS-CoV-2 in neonatal stool following remote maternal COVID-19.[22] Taken together these studies provide growing support for SARS-CoV-2 persistence.

SARS-CoV-2 can infect several cell types and in theory any infected cell is at risk of harboring persistent virus. The precise localization of SARS-CoV-2 persistence is unknown, but it is widely assumed that this occurs in tissues. This may or may not include immune-privileged sites as has been observed with other ribonucleic acid (RNA) viruses (e.g. Ebola).[23] Because tissue studies are generally impractical due to invasiveness of these procedures, there are now efforts to develop less-invasive measures to assess this. Some studies identified detectable SARS-CoV-2 RNA in plasma and stool during the early post-acute phase.[24] One recent study found that a large proportion of individuals with LC had at least intermittently detectable circulating antigen in the plasma for up to a year post-infection.[25] However, it remains unclear whether antigen can also persist in asymptomatic individuals, whether what is being detected represents remnants of a long extinguished infection or ongoing virus production from a long-lived reservoir or ongoing replication.

2.1 Hypothesis

We hypothesize that tissue viral persistence contributes to LC pathophysiology, and that administration of oral ensitrelvir to individuals experiencing LC will result in symptom reduction as well as improvement in biomarkers of inflammation in comparison to those receiving placebo.

2.2 Program Overview (LIINC Cohort)

This study will occur within the Long-term Impact of Infection with Novel Coronavirus (LIINC) cohort,[26] an observational study of post-COVID conditions that has been ongoing at UCSF since April 2020. Since that time, LIINC has been characterizing the natural history and biology of LC. The cohort has recruited over 700 individuals, of whom over 350 report mild-to-severe LC symptoms. The retention rate at 1 year is 89%. Individuals are evaluated at scheduled visits and biological specimens (plasma, serum, peripheral blood mononuclear cells (PBMCs)) are collected and stored. A subset of the cohort is referred for additional studies including advanced cardiopulmonary (echocardiogram, cardiopulmonary exercise testing, cardiac magnetic resonance imaging (MRI), pulmonary function tests, and tilt table tests) neurologic (neuropsychiatric testing, lumbar puncture), imaging (fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT), and tissue assessment (gut biopsy, lymph node fine needle aspiration). To date, the LIINC team has conducted over 3000 participant-encounters and have enrolled over 200 individuals into the more intensive protocols. LIINC has also described the relationship between LC and adaptive immune responses,[27] inflammation,[28-30] autoimmunity,[31,32] microbial translocation,[33] neurocognitive changes,[34,35] and cardiopulmonary physiology.[29,36] The LIINC team has for decades conducted investigator-initiated clinical trials as part of the associated HIV program (SCOPE) and has the capacity to implement the study described in this protocol using established procedures.

Potential participants will be required to enroll in LIINC, and the trial participants will be recruited directly from LIINC if they have had LC subsequent to an acute COVID episode

2.3 Risk/Benefit Assessment

The potential benefits to participants and society are likely to outweigh the minimal risks from participation in the study.

2.3.1 Potential Risks

Confidentiality. Participation in research may involve loss of privacy. Participants' records will be handled as confidentially as possible. All research records will be coded with a four-digit study identification (ID) code. Only the study investigators and their staff will have access to study records and test results. Study charts will be kept in a locked file cabinet in a locked office. Electronic data will be protected with a password and kept on a secure network. All collaborators will receive specimens only identified by the four-digit study ID. No individual identities will be used in any reports or publications resulting from this study.

Phlebotomy. Drawing blood from a vein may cause some discomfort, bleeding, or bruising where the needle enters the skin, and rarely, fainting or infection may occur. No more than 500 mL (2 cups) of blood will be drawn over any two-month period. This is within Red Cross guidelines. Risks of blood collection include anemia (low blood counts). Symptoms of anemia include tiredness, weakness, and dizziness. Participants will be checked for signs and symptoms of anemia at each visit, and CBC/diff will be checked as outlined in the schedule of events. If the investigator feels that a participant is at significant risk for anemia, the amount of blood collected will be reduced.

Nasal or oral swab. These collections may cause temporary discomfort.

Viral testing. Testing for viruses such as SARS-CoV-2 and viral hepatitis and testing may be performed as part of this study. Being tested for these infections may cause anxiety, regardless of the test results. Receiving a positive test result may cause a lot of anxiety. If the test result is negative, there is still the possibility that a participant could be infected and test positive at some time in the future. In addition, there is always the rare possibility that the test results could be wrong. Newly positive test results for SARS-CoV-2, HIV, hepatitis B, hepatitis C, and Tuberculosis (TB) infections are required to be reported to the Department of Public Health.

Drug side effects. No serious adverse events were observed in the published phase 2b data of ensitrelvir.[11] Possible side effects may include:

1) Headache, diarrhea or back pain. These appear to be mild with resolution following completion of drug.

2) Allergic reaction, including rash, may occur after any novel antiviral medication.

3) Transient increases in blood HDL and triglycerides while on antiviral therapy.

3) Participants may experience return or change in Long COVID symptoms following cessation of study drug as has been anecdotally reported with other direct antiviral use in Long COVID.

5) Unanticipated AEs. These are not all the possible side effects.

2.3.2 Protection Against Risk

Confidentiality. Participants' records will be handled as confidentially as possible. Participants will be assigned a unique four-digit study ID code that will appear on all specimens and in our database. All biologic specimens and clinical data obtained from this study will be linked to this code and not to personal identifying information (e.g., name, social security number, medical record number). A key

which will link the four-digit code to the personal information will be maintained on a secure server only accessible to designated study staff and maintained by the PI. Lab personnel and database programmers will have access only to the coded number and the participant's date of birth; no other personal identification information will be available to them. Collaborators will receive specimens only identified by the four-digit study ID. No individual identities will be used in any reports or publications resulting from this study.

Phlebotomy. Relevant personnel are trained and certified phlebotomists. We will stay within Red Cross Guidelines of less than 500 mL every two months. Participants will be checked for anemia at each study visit. If the investigator feels that an individual is at significant risk for anemia, the amount of blood collected will be reduced. If the study participant's hemoglobin falls below 9 grams/deciliter (g/dl) or HCT falls below 27%, we will draw 5 mL of blood to check safety labs such as Hgb and HCT. Other than the blood required to check safety labs, the participant will not have more blood drawn until the Hgb rises above 9 g/dl or the HCT rises above 27%.

2.3.3 Benefits to Participants

There is currently no accepted treatment for LC. Participants should not anticipate a personal benefit from participation in the study. However, it is possible that if the therapeutic intervention has the hypothesized effects, participants could experience relief from LC symptoms. Furthermore, it is possible that participants might benefit in general from engagement in research.

2.3.4 Benefits to Society

The identification of a treatment for LC symptoms could benefit the likely millions of individuals suffering from this condition.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of the study is to evaluate the safety and tolerability of oral ensitrelvir in individuals who are experiencing Long COVID.

3.2 Secondary Objectives

Secondary objectives include the following:

- Describe the ability of ensitrelvir to affect virologic markers of chronic SARS-CoV-2 infection.
- Describe the ability of ensitrelvir to improve PASC outcomes.
- Describe the ability of ensitrelvir to reduce post-COVID inflammation.

4 STUDY DESIGN

4.1 Study Overview

This is an exploratory, 1:1 randomized, double-blind, placebo-controlled study with optional post-blinding placebo to treatment to assess the safety and efficacy of ensitrelvir to treat LC.

The study will enroll approximately 40 participants who meet the WHO LC criteria (defined above in protocol synopsis). Participants will be enrolled at a single center and randomized 1:1 to receive ensitrelvir fumaric acid (S-217622) given orally 375 mg on day 1 followed by 125 mg daily for 4 additional days or placebo. Randomization will be stratified by duration of infection (<6 months and ≥6

months) at Day 0. Evaluations will take place at baseline and at timepoints up to 60 days after initiation of study drug.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- Ensitrelvir Fumaric Acid (S-217622) given orally 375 mg on day 1 followed by 125 mg daily for 4 additional days
- Placebo

The total duration of subject participation will be approximately 2 months.

5 CRITERIA FOR EVALUATION

5.1 Efficacy Endpoints

- The change in patient-reported outcomes (PROs) PROMIS-29 score from Baseline to D30.
- Change PROMIS-29 score in those with symptoms at Baseline and at D10, and D30.
- Change in EuroQoL Quality of Life between Baseline and at D10, D30, and D60.
- Change in other assessments (neurocognitive assessment and 6MWT performance) between Baseline and at D10.
- Change in other assessments (DASI, Dysautonomia assessment) between Baseline and at D10, and D30.
- Percentage of participants with no detection of SARS-CoV-2 plasma remnants (i.e., viral detection by reverse transcriptase-polymerase chain reaction ((RT-PCR) and Spike protein fragments) compared to baseline at D10 and D30 post ensitrelvir administration.
- Proportion with reduction in inflammatory markers (e.g., interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha) at Baseline and at D10 and D30.

5.2 Safety Evaluations

- Number of participants with adverse events (AE)s (Treatment Emergent Adverse Events (TEAE)s, Serious Adverse Events (SAE)s, and Adverse Events of Special Interest (AESI)). The primary outcome will be the number of individuals in each group experiencing a severe (Grade 3 or greater) AE thought to be possibly, probably, or definitely related to study treatment throughout the study duration.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a history of SARS-CoV-2 infection who meet the case definition for Long COVID, and who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Age ≥18 and <70 years at Screening.
2. Enrolled or willing to enroll and complete at least 1 visit in the UCSF Long-term Impact of Infection with Novel Coronavirus (LIINC) study. Any adult who has been infected with SARS-CoV-2 or has ever received or is eligible to receive a SARS-CoV-2 vaccination, and who is able to provide written informed consent, is eligible to participate in LIINC.

3. History of at least one SARS-CoV-2 infection, defined as report of a positive nucleic acid amplification test (NAAT) and/or a positive SARS-CoV-2 antigen rapid diagnostic test (RDT). Written proof of the test will be requested but is not required as long as the participant attests to the positive test.
4. Clinical evidence of Long COVID, as confirmed by the investigator's assessment.
    a. At least two moderate symptoms or at least one severe symptom as assessed by the study team (see list) that are new or worsened since the time of a SARS-CoV-2 infection, not known to be attributable to another cause upon assessment by the PI. At least two symptoms from those listed here must be present: systemic symptoms (e.g., fatigue, chills, post-exertional malaise), neurocognitive symptoms (e.g., trouble with memory/concentration ("brain fog"), headache, dysautonomia/postural orthostatic tachycardia syndrome, dizziness, unsteadiness, neuropathy, sleep disturbance), cardiopulmonary symptoms (e.g., chest pain, palpitations, shortness of breath, cough, fainting spells), musculoskeletal symptoms (e.g., muscle aches, joint pain), gastrointestinal symptoms (e.g., nausea, diarrhea). Although other symptoms (e.g., skin rash, hair loss, mental health symptoms, trouble with smell/taste, genitourinary symptoms) will be recorded and tracked, at least two core symptoms listed above must be present. Note: the two symptoms can be from within the same category (for example, brain fog and headache).

        AND

    b. Symptoms must have been present for at least 60 days prior to screening. Symptoms that wax and wane must have been initially present at least 60 days prior to screening.

        AND

    c. Symptoms must be reported to be at least somewhat bothersome and to have an impact on quality of life and/or everyday functioning.

        AND

    d. At least 90 days have elapsed since the most recent suspected or confirmed SARS-CoV-2 infection and the time of screening. Note: suspected infections will be determined based upon assessment by the study investigators.
5. Not currently hospitalized.
3. Body mass index (BMI) 18 to 50 kilograms/meter squared (kg/m2), inclusive, at the time of screening.
6. In otherwise stable health, as assessed by the investigator within 28 days prior to Screening, based on medical history, physical assessment, laboratory findings, and vital signs.
7. For Male participants,

    Participants with partners that are women of childbearing potential (WOCBP) are strongly advised to inform their partners and must agree to use effective contraception from study entry (defined as D0) through 14 days after the last dose of study intervention. Effective methods of contraception are described in Appendix 2. Participants with pregnant partners must agree to use condoms during vaginal intercourse from study entry (defined as D0) through 14 days after the last dose of study intervention administration. Participants assigned male sex at birth must agree to refrain from sperm donation from study entry through 14 days after the last dose of study intervention administration.
8. For Female participants,

A female participant is eligible to participate if she is not pregnant or breastfeeding, and the following conditions applies:

- Is not a WOCBP

OR

- All of the following apply:

4. Is a WOCBP and using a contraceptive method that is effective as described in Appendix 2 from –21 days from study entry (defined as D0), during the study intervention period, and for at least 14 days after the last study intervention administration. She must also agree not to donate her eggs (ova, oocytes) for the purpose of reproduction from study entry (defined as D0), during the study intervention period, and for at least 14 days after the last study intervention administration. (Of note, participants taking hormonal contraception will be informed that there may be a possibility of decreased efficacy or contraceptive failure while on study drug.)
    - ✓ A WOCBP must have a negative urine pregnancy test within 24 hours before the first dose of study intervention. If a urine pregnancy test cannot be confirmed as negative (e.g., an ambiguous result), a serum pregnancy test must be negative.
9. Willingness and ability to comply with the study protocol. This includes reliable transportation and sufficient time to attend all visits.
10. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

5. Previously received COVID-19 convalescent plasma treatment or a SARS-CoV-2-specific mAb within 60 days prior to planned Day 0 or plan to receive such treatment before exiting the study.
1. Previously received a SARS-CoV-2 antiviral within 60 days prior to planned Day 0 or plan to receive such treatment before exiting the study.
2. Plans to receive any investigational or approved vaccine or booster for SARS-CoV-2 within 60 days prior to planned Day 0 or before Day 30 following planned Day 0.
3. Active cardiovascular disease, defined as known:
    a. Myocardial infarction within 90 days of screening

       OR

    b. Coronary artery bypass procedure within 90 days of screening

       OR

    c. Current heart failure with reduced ejection fraction (<45%)

       OR

    d. Current pulmonary arterial hypertension.
4. Known stroke within 3 months prior to planned Day 0.
5. Known active bacterial, fungal, viral, or other infection besides SARS-CoV-2 requiring treatment within the 28 days prior to planned Day 0 and meeting criteria for systemic involvement upon review by the PI. Note: Mild or limited infections such as uncomplicated urinary tract or yeast infections, sexually transmitted infections, and mild dermatophyte infections may be reviewed with the study PI and/or Safety Monitoring Committee chair, but are not exclusionary.

6. Major surgery within 6 months prior to planned Day 0 or planned major surgery during the first 60 days following planned Day 0.
7. History of unplanned hospitalization for >24 hours within 28 days prior to Screening.
8. Active Hepatitis B (Hep B) infection (defined as Hep B surface antigen (sAg) positive). Note: A known positive Hep B core antibody (cAb) in the absence of positive sAg is not considered exclusionary.
9. Active Hepatitis C (Hep C) infection (defined as Hep C Ab positive or indeterminate with detectable Hep C RNA). Note: Those with cured Hep C (Ab positive or indeterminate but negative Hep C RNA) will remain eligible.
10. Laboratory abnormalities at Screening, specifically including:
    a. ANC <1500 per mm^3
    b. Platelet count <100,000 per mm^3
    c. AST/ALT > 1.5x ULN
    d. CrCl < 30 mL/min
    e. Hemoglobin <9 g/dL
11. Known HIV infection.
12. End stage kidney disease requiring dialysis.
13. Severe hepatic impairment (Child-Pugh Class C).
14. Moderate or severe immunocompromise, according to the current NIH COVID-19 Treatment Guidelines as of March 6, 2023. The detailed list is in Appendix 2, and includes the following: (a) receiving active treatment for solid tumor or hematologic malignancy, including use of systemic chemotherapy for treatment of cancer within the year prior to screening, (b) prior solid-organ transplant with active immunosuppressive therapy, (c) CAR-T cell therapy or hematopoietic cell transplant, on immunosuppressive therapy or transplant within the prior 2 years, (d) primary immunodeficiency syndromes, advanced or untreated HIV infection (any HIV infection is exclusionary), (f) on active high-dose corticosteroids (i.e., >= 20mg prednisone or equivalent daily per day for >= 2 weeks) at the time of screening.
15. Known prior diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), preceding and not related to SARS-CoV-2 infection and not worsened since SARS-CoV-2 infection.
16. Known prior diagnosis of dysautonomia, preceding and not related to SARS-CoV-2 infection and not worsened since SARS-CoV-2 infection.
17. Pregnant, breastfeeding, or unwilling to practice birth control abide by the contraception requirements outlined in the inclusion criteria.
18. Participation in a clinical trial with receipt of an investigational product within 28 days or 5 half-lives (whichever is longer) prior to planned Day 0. The following exceptions will apply:
    a. If the investigational product is a SARS-CoV-2-specific monoclonal antibody, convalescent plasma, or equivalent (e.g., IVIG), refer to Criterion #1 (60 days must have elapsed prior to planned D0).
    b. If the investigational product is a PET imaging tracer that is not expected to have significant biological activity, has a biological half-life less than 24 hours and radiological half-life less than 6 hours; more than 14 days must have elapsed between receipt of the tracer and planned Day 0.
19. Current alcohol or illicit drug use as determined by the investigator to preclude participation.

20. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
21. Study site personnel directly affiliated with the study or family of directly involved personnel.
22. Known allergy to any components used in the formulation of the intervention.
23. Current use of or anticipated need for any medications prohibited with study intervention, as described in Appendix 3.
24. Participants who have used any of the following drugs within 14 days prior to enrollment:
    - ✓ Strong cytochrome P450 (CYP) 3A inducer
    - ✓ Products containing St. John's wort

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies unless deemed necessary by a medical provider with the exception of exclusionary medications as listed above. Initiation of over-the-counter supplements will be discouraged.

7.1 Allowed Medications and Treatments

Participation in the study will not interfere with the participant's standard of care. Routine or standard of care vaccinations (such as influenza, pneumococcus, etc.) are allowed and will be documented in the CRFs; wherever possible these will be spaced greater than or equal to two weeks from study visits.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

The 40 participants will be randomized, 20 to ensitrelvir and 20 to placebo. The randomization will be stratified by duration of COVID-19 related symptoms (< 6 months vs. ≥ 6 months). Within the strata, randomization will use permuted blocks of 3 and 6 enrollees with equal likelihood. Following study unblinding, participants in the placebo arm will be given the option to receive ensitrelvir with similar safety and efficacy evaluations as in the primary study population.

8.2 Blinding

Treatment assignments will be blinded to the investigator, subjects, and all clinical and research staff for the entire study, except for designated pharmacy staff or delegate who will remain unblinded to prepare the study drug or placebo.

Unblinding can occur at any time for a medical emergency or any other significant medical event and when a treatment decision is contingent on knowing the subject's treatment assignment. When possible, the investigator or delegate should discuss with Shionogi prior to unblinding. The date and reason for unblinding must be recorded and Shionogi will be notified.

The Independent Medical Monitor (IMM) may review any unblinded clinical analysis during the study and provide the recommendations to the Investigator. Subject level unblinded data will not be shared with the Investigator until the end of the trial.

8.3 Formulation of Test and Control Products

Ensitrelvir and placebo will be provided by the manufacturer who will be responsible for ensuring that ensitrelvir and placebo are manufactured in accordance with applicable current Good Manufacturing Practice (GMP) regulations and requirements.

8.3.1 Packaging and Labeling

The study drugs will be labeled according to the requirements of local law and legislation. The study drugs will be dispensed according to GCP by the clinical site's pharmacy in accordance with the site's standard operating procedures (SOPs).

8.4 Supply of Study Drug at the Site

The manufacturer (or designee) will ship ensitrelvir and placebo to the investigational site's clinical research pharmacy. The initial ensitrelvir and placebo shipment will be shipped after site activation (i.e., all required regulatory documentation has been received by Shionogi and a contract has been executed). Subsequent ensitrelvir and/or placebo shipments will be made after site request for resupply.

8.4.1 Dosage/Dosage Regimen

Ensitrelvir is administered orally over 5 days. On the first day, 3 125 mg pills (total 375 mg) will be taken orally followed by 4 additional days of one 125 mg pill per day. Placebo is also administered orally, with three pills being taken on the first day followed by 1 pill daily for 4 additional days. Details regarding the preparation and administration of the study drugs are provided in the pharmacy manual.

There is no adjustment for weight, age, meals, or other factors.

8.4.2 Dispensing

Participants will receive the full 5-day course of treatment at the time of the D0 visit. Participants will receive a treatment log to document the time that each dose was taken. Compliance will be assessed during the D1 and D4 phone follow-up and participants will be asked to return their treatment log at the in-person follow-up visit.

8.4.3 Administration Instructions

The date and time of each dose will be reported to the study team by the participants and to be started on the day designated by the study team.

8.5 Supply of Study Drug at the Site

The investigator will delegate responsibility for drug receipt, storage, accountability, and disposition to the clinical site's research pharmacist. The clinical site's pharmacist will maintain an inventory record of the study drugs received, stored (in a secure restricted area), and dispensed. Study drugs and placebo will be provided to study participants only.

8.5.1 Storage

Ensitrelvir pills and placebo will be shipped from the manufacturer or manufacturer resources to the clinical site's pharmacy. The study medication will be stored at room temperature.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing for each subject will be maintained on an ongoing basis by the research pharmacist. The number of study drug dispensed will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.7 Measures of Treatment Compliance

Since this study involves a short course of oral antiviral pills, participants will be asked to keep a written log of the timing of each oral dose to be provided to the study team once they complete the treatment or placebo course.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1. The Schedule of Events will serve as the final resource for guiding study activities.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies taken within 28 days prior to Screening will be documented. Dose, route, unit frequency of administration, and indication for administration and actual or estimated dates of medication will be captured.

Details regarding all SARS-CoV-2 vaccinations and/or boosters, as well as COVID-specific therapies, will be collected.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination at Screening will be performed by a qualified study clinician (physician, nurse, or physician assistant). Qualified staff (MD, DO, NP, RN, or PA) may also complete the abbreviated (targeted) physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs and Oximetry

Weight, body temperature, blood pressure, pulse, respirations, and oximetry on room air will be performed after resting for 5 minutes or more as indicated in the Schedule of Events (SOE). Vital signs in the lying, seated, and standing positions may also be performed.

9.1.6 Questionnaire-based Measurements

Long COVID Symptom Assessment. Signs and symptoms related to Long COVID will be reviewed at all visits, using forms such as the LIINC CRFs. At D0 and all subsequent visits, all grades of signs and symptoms that are newly developed or changed since the previous visit must be recorded. Duration (start and stop dates), severity/grade, outcome, treatment, and relation to study drug will be recorded on the case report forms. All clinical events and new diagnoses or changes in diagnoses should be recorded.

Other Symptom Assessment (AE Review). At D0 and all subsequent visits, all grades of signs and symptoms that are newly developed or changed since the previous visit will be recorded. Symptoms not previously experienced as part of Long COVID, or which have worsened beyond the degree previously experienced by the participant, will be considered to represent adverse events and recorded as such. For these symptoms, duration (start and stop dates), severity/grade, outcome, treatment, and relation to study drug will be recorded on the case report forms. All clinical events and new diagnoses or changes in diagnoses should be recorded.

Quality of Life. Quality of life (QoL) will be obtained using the EQ-5D-5L scale which is part of the LIINC CRFs. This will include the 100-point visual analogue scale (VAS).

PROMIS-29. The PROMIS-29 form will be completed as outlined in the schedule of events. This scale measures self-reported health, using a collection of short forms assessing fatigue, physical function, anxiety, depression, pain, sleep disturbance, and ability to participate in social roles and activities. The questionnaire uses a computer interface over approximately 5 minutes. It is available in English and Spanish. This instrument has been used for ME/CFS and Long COVID.

Patient Global Impression of Change (PGIC). The self-report measure Patient Global Impression of Change (PGIC) reflects a patient's belief about the efficacy of their treatment. We will use a modified PGIC scale which has been used to study pain syndromes and has been employed in other Long COVID clinical trials. It is a common data element developed by the National Institutes of Mental Health.

Everyday Cognition Form (ECOG). The ECOG is performed in the parent LIINC study and asks participants to self-rate issues with cognition. It is a patient-reported measure for neurocognitive symptoms.

Neurocognitive Assessment. A neurocognitive assessment such as the NIH Toolbox or similar assessment will be administered as outlined in the schedule of events. Neurocognitive assessments can assess motor, emotional, sensory, and cognitive function. It is available in English and Spanish and is administered using an iPad or computer.

Duke Activity Status Index. The Duke Activity Status Index is a patient-reported estimate of functional capacity, maximal oxygen consumption (VO2 max) and maximum metabolic equivalent of tasks (METs). The DASI questionnaire produces a score between 0 and 58.2 points, which is linearly correlated with a patient's VO2 max and METs, as measured from cardiopulmonary exercise testing (CPET). It inquires about a person's ability to perform self-care, walk, climb stairs, run, do house and yard work, engage in sexual intercourse, and perform moderate recreational activities.

Dysautonomia Assessment (e.g., COMPASS-31 or similar Scale). The Composite Autonomic Symptom Score (COMPASS)-31 is an assessment of autonomic dysfunction. This abbreviated questionnaire provides a quantitative measure of autonomic symptoms that otherwise might not be adequately recorded using the above instruments. It includes measures of orthostatic intolerance, vasomotor and secretomotor symptoms, GI and urinary symptoms, syncope, and genitourinary symptoms. We will utilize this questionnaire or a similar instrument to determine the impact of treatment on post-COVID autonomic dysfunction.

DSQ-PEM (Short Form). The DePaul Symptom Questionnaire (DSQ) post-exertional malaise (PEM) questionnaire will be used to assess post-exertional malaise at baseline, and again at the primary endpoint as specified in the schedule of events.

WHO-DAS (Short Form). The World Health Organization Disability Assessment Schedule 2.0 questionnaire asks about difficulties due to health conditions. Health conditions include diseases or illnesses, other health problems that may be short or long lasting, injuries, mental or emotional problems, and problems with alcohol or drugs. We will utilize this questionnaire at baseline, and again at the primary endpoint as specified in the schedule of events.

6 Minute Walk Test. A 6MWT will be performed by the research team.

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

This is a study of Long COVID symptoms. As a result, symptoms experienced prior to receipt of the study intervention will be documented, and a symptom will only be considered to represent an AE if its quality or severity differs from or exceeds that which was previously experienced by the participant.

9.1.8 Participants with Positive COVID Tests During the Study

Due to the ongoing pandemic, it is anticipated that some individuals may test positive for COVID-19 during the course of the study. If an individual exhibits acute COVID-19 symptoms during the study, we will arrange for or navigate to testing. If an individual tests positive for SARS-CoV-2 during the course of the study, we will leverage existing testing and treatment programs in our hospital system and/or geographic region to navigate the participant to care. In addition to navigation for clinical care, all participants who test positive during the study will be referred to a home-based research collection protocol for more frequent sampling.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), and serum C-reactive protein (CRP) determinations for assessment of systemic evidence for infection and/or inflammation. These measurements will be performed as outlined in the Schedule of Events.

9.2.2 Blood Chemistry Profile

Blood will be obtained and sent to the site's clinical chemistry lab for determination of serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, direct bilirubin, and albumin. These measurements will be performed as outlined in the Schedule of Events.

9.2.3 Pregnancy Testing

A urine or serum pregnancy test will be obtained from female subjects who are of childbearing potential prior to their participation in the study. These measurements will be performed as outlined in the Schedule of Events.

9.2.4 Hepatitis B Testing

Participants will undergo Hep B surface antigen testing at Screening, if they have not had such screening within the preceding 6 months. Individuals with active Hep B will be excluded. This is defined as detectable Hep B surface antigen. Individuals without detectable Hep B surface antigen will remain eligible, regardless of their vaccination or antibody status (e.g., a positive Hep B cAb or negative Hep B sAb is not exclusionary).

9.2.5 Hepatitis C Testing

Participants will undergo Hep C antibody testing at Screening, if they have not had such screening within the preceding 6 months. Individuals with active Hep C, defined as positive or indeterminate antibody with the presence of Hep C RNA, will be excluded. Positive or indeterminate Hep C Ab in the absence of Hep C RNA is not exclusionary.

9.2.6 SARS-CoV-2 PCR or Antigen Testing

Swabs of the anterior nares will be performed to assess for SARS-CoV-2 shedding as outlined in the Schedule of Events.

9.3 Research Laboratory Measurements

9.3.1 Plasma Measures of Viral Persistence

Plasma levels of SARS-CoV-2 antigens (Spike, nucleocapsid, etc.) may be performed in collaboration with research laboratories to evaluate the effect of antiviral therapy on measures of viral persistence.

9.3.2 Immunophenotyping

Single- or multi-plex platforms will be used to measure targeted biomarkers previously shown to be elevated in Long COVID, which may include interleukin-6, tumor necrosis factor-alpha, monocyte chemoattractant protein-1, and interferon-gamma induced protein 10. In addition, we may use high-throughput platforms such as Olink proteomics to conduct within-person and/or between-group comparisons in order to identify pathways that are altered with treatment, or that differ between those with and without improvement during the study period. Additional measures of cellular and humoral immunity may also be performed.

9.3.3 SARS-CoV-2 Genotyping

Genotyping may be performed on any samples collected during the study in which SARS-CoV-2 virus is recovered. Participants with a positive test at the time of screening are not eligible for the study. Participants with suspected acute SARS-CoV-2 infection or who report rebound symptoms at the time of an in-person follow-up visit will undergo a SARS-CoV-2 test and if positive the sample will be banked for genotyping analyses.

9.4 Biospecimens

Within the UCSF LIINC infrastructure, initial sample processing is performed on peripheral blood units which are then transported by a staff member or courier to a biorepository, which include the Molecular and Cellular Core (formerly Core Immunology Laboratory) and the UCSF Specimen Processing and Banking Subcore (formerly AIDS Specimen Bank). All samples are tracked through laboratory information systems and managed with sample storage inventories, monthly reports, and institutionally managed data storage and back-up systems. All biospecimens will eventually be stored long-term via the UCSF Specimen Processing and Banking Subcore (formerly AIDS Specimen Bank), where the existing 50,000-specimen LIINC repository is currently housed and managed. Specimens are stored in

ultra-low temperature freezers (including liquid nitrogen) with back-up power systems, in which temperature is monitored by a programmable scanning alarm system wired to the university's telephone system. After completion of the primary analyses, samples may be stored indefinitely via the LIINC biorepository for use on other COVID-related pathogenesis studies (as addressed in the consent form), at the discretion of the PI.

10 EVALUATIONS BY VISIT

The details of the visit schedule are outlined below. The Schedule of Events will be the final guide for what events are to occur at each specific study visit.

10.1 Screening Visit (D-28)

Following the informed consent discussion and signed informed consent form, the Screening Assessment will be performed. The inclusion and exclusion criteria will be reviewed with the participant. Screening labs, medical history, and the details of concomitant medications will be obtained. Details of the participant's COVID-19 history (including COVID-19 treatments) will be reviewed and/or confirmed.

The Screening Assessment will include a physical assessment by a study clinician (physician, nurse, or physician assistant). Other assessments as outlined in the Schedule of Events will be performed.

Laboratory tests as outlined in the Schedule of Events will be performed. Laboratory tests or other assessments performed for a clinical indication (not exclusively to determine study eligibility) may be used for screening values even if the studies were performed before informed consent was obtained. An individual who recently had laboratory tests performed as part of routine clinical care need not repeat these tests if they are within the window for the test (e.g., within 6 months for hepatitis testing). For example, someone with recent Hepatitis B or C or HIV testing may not need to have the test repeated, as indicated in the Schedule of Events. However, these measurements may be repeated at the discretion of the PI.

Screening evaluations should be completed within 28 days of planned D0. If more than 28 days lapse, screening procedures may be repeated to re-confirm eligibility prior to D0. At a minimum, this will include the metabolic panel, complete blood count, and pregnancy testing. Laboratory tests that are not expected to change (such as coagulation studies or viral hepatitis status) are not required to be repeated but may be at the discretion of the PI.

Rescreening: A participant who does not meet all eligibility criteria at the time of Screening may be given the opportunity to rescreen for the study at a later date.

10.2 Baseline Evaluation (D-21 to D0)

An additional visit will be performed following Screening, at least 7 days after the screening visit and prior to D0. The target date for this visit is within 7 days prior to the scheduled date of study product administration, although up to 21 days is acceptable. The purpose of this visit is to make additional assessments prior to the intervention, to establish baseline symptomatology and measurements. This will include a symptom assessment and various clinical assessments as outlined in the Schedule of Events. Certain real-time laboratory tests will be obtained to establish a baseline.

Note, after biospecimen collection has occurred, the participant may immediately move on to the intervention visit (medications can be dispensed after the baseline evaluations are complete).

10.3 Intervention (D0)

Participants who screen into the study and complete baseline measurements will be randomized to receive either ensitrelvir or placebo. D0 refers to the day that the participants start taking oral study medication.

Note: During the D0 encounter or at the reminder call prior to that visit, a team member will confirm that there have been no major changes to the participant's health since the baseline visit that would affect eligibility. If there have been major changes, this will be discussed with a study clinician prior to product administration to confirm whether or not it is acceptable to proceed. If the Baseline and Entry visit are on the same day (i.e., combined), blood will be collected only once.

Note: For persons of childbearing potential, a negative pregnancy test result must be obtained prior to dispensing the study product.

10.4 Telephone Follow-ups (Phone Follow-Up, D1, D4)

The participant will be contacted by telephone to check on the overall response to the first and last dose of medication. The Phone FU D1 visit will focus on determining whether there have been any adverse events from the study product. The Phase FU on D4 will focus on determining whether there have been any adverse events from the study product, as well as whether there has been any short-term efficacy.

10.5 In Person Follow-up Visits (D10, D30)

In-person follow-up visits will occur as outlined in the Schedule of Events. At each visit, vital signs will be collected, and the participant will undergo a targeted physical assessment as needed. They will complete clinical assessments as outlined in the Schedule of Events. Laboratory tests will be ordered and biospecimens collected and stored for later testing as per the Schedule of Events.

10.6 Telephone or In Person End-Of-Study Visit (EOS D60)

The participant will be contacted by telephone or by in person interview with study staff to undergo clinical questionnaire assessments as outlined in the Schedule of Events.

10.7 Unscheduled (Interim) Visits

An unscheduled (or interim) visit is defined as any visit not pre-specified in the study protocol. An unscheduled visit may be scheduled in order to assess a participant in person, repeat a laboratory test, or to collect additional biospecimens at the discretion of the PI.

10.8 Study Discontinuation Visit

If a subject withdraws or discontinues from the study, they should complete a Study Discontinuation Visit. If D0 has occurred, this visit should at a minimum include AE assessment and documentation of the reason for discontinuation or withdrawal. Whenever possible, the study events for the Study Discontinuation Visit should be as follows:

- Prior to D0: No further assessment needed.
- Between D0 and D10: Complete D10 assessments.
- After D10: Complete D30 assessments (even if D30 has elapsed).

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one

of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

Suspected Adverse Reaction (SAR) – any AE for which there is a reasonable possibility that the drug caused it. It implies a lesser degree of certainty about causality than **adverse reaction** (any AE caused by a drug).

An unexpected AE or unexpected SAR is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

11.1.1 AE Severity

The National Institutes of Health Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 (July 2017) will be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

11.1.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2 below.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment

Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE) or Serious SAR

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

A **life-threatening AE or life-threatening SAR** is an occurrence that places the patient or subject at immediate risk of death. It does NOT include an AE or SAR that, had it occurred in a more severe form, might have caused death.

SUSARS are fatal or life threatening serious unexpected SARs. The team must notify the FDA as soon as possible, but no later than 7 calendar days after the initial receipt of information. Serious non-fatal or non-life-threatening SARs will be reported to Shionogi as soon as possible, but no later than 15 calendar days after initial receipt of information.

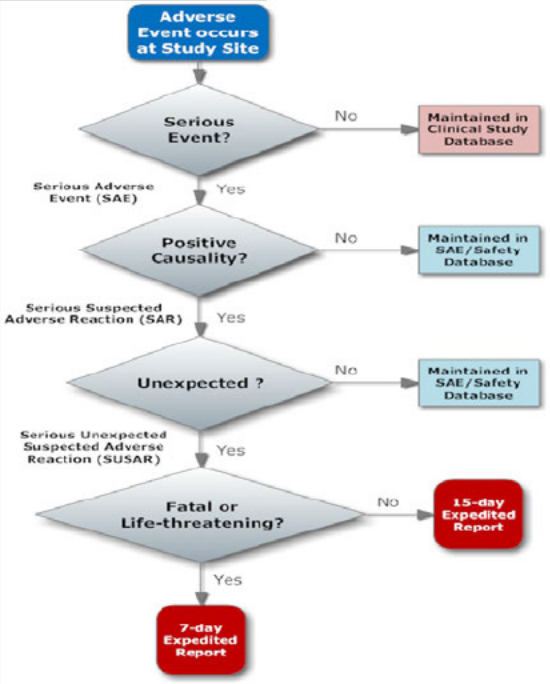
11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

Shionogi, the manufacturer of the IP, shall be notified within 24 hours of study team investigator awareness of an SAE. In addition, an AE master list shall be sent to Shionogi Safety monthly. The detail should be described separately in PV agreement.

FDA IND Expedited Safety reporting Requirements



Additional FDA IND 15-day expedited reporting includes new safety findings from other clinical trials, findings from in vitro and animal testing, and increased rate of occurrence of serious SARS.

11.3 Liver Toxicity

In addition to excluding participants with screening liver test abnormalities as outlined above and with a history of severe liver disease, we will perform post-treatment liver function test monitoring as outlined in the Schedule of Events. We will follow the below recommendations adapted from the Guidance for Industry Drug Indiced Liver Injury Premarketing Clinical Evaluation document available at https://www.fda.gov/media/116737/download.

- An increase of serum AST or ALT to >3x ULN will be followed by repeat testing within approximately 72 hours to confirm the abnormalities and to determine if they are ncreasing or decreasing. Symptoms will be assessed. If symptoms persist or repeat testing shows AST or ALT >3x ULN, close observation (see below) will be continued until the abnormalities resolve.
- Since the participant would have already completed dosing, close observation includes repeating liver enzyme and serum bilirubin tests at least weekly until the abnormalities stabilize in the opinion of the investigator. In addition, the study team will obtain a detailed symptomatic history, history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets. The team will assess for alternative causes of hepatitis (including viral, autoimmune, alcoholic, ischemic, and related to biliary tract disease). Additional tests to evaluate liver function (e.g., INR) will be obtained and the participant may be referred for hepatology consultation.

11.4 Study Drug Overdose

If the study team learns of a participant overdosing on drug (defined as taking more than double the specified dose in one administration), the participant will be asked to come in for safety laboratory

monitoring. This will include liver toxicity monitoring, symptom assessment, and any other monitoring deemed indicated by the study investigator.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation

A subject may be discontinued from study treatment at any time if the subject or the investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Manufacturer request for early termination of study

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit (as outlined above) as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for study discontinuation procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject or the investigator feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to D60) should have an early discontinuation visit. Refer to Section 10 for early termination procedures.

12.3 Replacement of Subjects

Subjects who withdraw from the study prior will be replaced at the discretion of the investigator.

12.4 Lost to Follow Up

A subject will be considered LTFU if s/he repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether the subject wishes to and/or should continue in the study.
- Before a subject is deemed LTFU, the investigator or designee must make every effort to regain contact with the subject (at a minimum, 2 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's source documents.

Should the subject continue to be unreachable, s/he will be considered to have withdrawn from the study

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety, and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Investigator will determine if a protocol violation results in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by the Investigator or delegate. A copy of the form will be filed in the site's regulatory binder and in the Investigator's files.

14 DATA SAFETY MONITORING

Safety Monitoring Committee (SMC): We will develop an independent SMC prior to the initiation of the study. We expect that the committee will be composed of at least 3 independent individuals from the scientific community. These individuals will be selected based on their expertise in either the clinical management of SARS-CoV-2 infection and/or their expertise with other viral infections and clinical studies. The committee will be chaired by a senior investigator who has experience in the regulatory aspects of clinical studies.

Approximately 4 months after enrollment of the first participant and then every 4 months thereafter, the SMC will review accrual (including screening and enrollment), AE summaries, including all reported Grade ≥3 AEs, retention of participants including off-study rates.

In addition to the regularly scheduled reviews, a safety review will be conducted by the SMC for any of the following criteria:

- A participant develops a Grade 3 or 4 AE that is definitely, probably, or possibly related to the study product, as determined by the investigator.
- A participant develops an SAE that is deemed possibly, probably, or definitely related to the study product, as determined by the investigator.

If the above criteria are met, the PI will request a review by the SMC, to be held within 3 business days of learning of the event.

In addition, enrollment into the study will be paused (defined as newly scheduled screenings or new dispensing of study product) until the SMC review has taken place and a determination has been made that enrollment can resume. In addition, administration of study products will be paused until a course of action is recommended by the SMC. Previously scheduled screening, baseline, and follow-up visits can continue during this period of review as these are not expected to affect the safety of participants who have not yet received the study IP or who have already received the study IP. However, new study IP will not be dispensed while the study is paused.

The SMC will recommend, based on the results of the review, whether the study can proceed as planned, proceed with modifications, or should be discontinued.

The SMC will review progress towards pre-specified benchmarks of enrollment and retention of participants, completion of study procedures, and collection of viable samples. If progress towards any benchmark is not adequate, as determined by the SMC, the SMC will recommend protocol modification if necessary.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

Eligible patients who are randomized into the study and receive at least one dose of the study drug during the randomized stage of this study will comprise the modified intent to treat (mITT) population and will be the primary analysis population for all analyses.

Per protocol analysis will be based on description of the results per study group excluding anyone who has received a non-study COVID antiviral therapy during the trial period.

Exploratory analyses will include mITT population with addition of participants original assigned to placebo who opt to undergo ensitrelvir therapy following unblinding in the optional cross-over stage. As this is exploratory, results of the primary and secondary endpoints from the initial randomized cohort may be reported prior to completion of the cross-over option.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by treatment group.

15.3 Analysis of Primary Endpoint

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. Adverse events between groups will be summarized by proportions with associated 95% Clopper-Pearson confidence intervals and will be compared using a two-sided 0.05 level Fisher exact test.

15.4 Analysis of Secondary Endpoints

The key secondary outcome will be the change in the PROMIS-29 scores from baseline to D10 follow up. This will be analyzed from the mITT population using a linear regression model for follow-up PROMIS-29 with terms for the baseline value, randomized treatment and randomization strata (<26 v.

>= 26 weeks of symptoms) – an analysis of covariance model (ANCOVA) and tested using a two-sided 0.05 level test.

Continuous secondary events (e.g., IL-6) will be compared using the ANCOVA approach. Antigen detection will be summarized by proportion with 95% Clopper-Pearson confidence intervals and will be compared using a two-sided 0.05 level Fisher exact test

Primary and secondary endpoint analyses will be performed on the mITT of the pre-unblinded randomized portion of the study. Exploratory analyses will be performed including the roll-over participants from placebo to treatment group. Primary and secondary endpoint analyses may proceed prior to completion of this optional roll-over study stage.

15.5 Sample Size and Randomization

This is an exploratory study, and the primary results will be descriptive. We will use ensitrelvir as a probe to better understand LC biology and determine whether a signal is present to warrant further study.

The study will enroll 40 participants who meet the WHO LC criteria. Participants will be enrolled at a single center and randomized 1:1 to receive ensitrelvir or placebo. Randomization will be stratified by duration of infection (<6 months and ≥6 months) at Day 0.

For PROMIS-29, assuming a standard deviation (SD) of the within-person difference of 10, then the planned sample size will have 80% power to detect a difference of 11.2 on a two-sided 0.05 level test.

For biomarkers, such as IL-6, we will be able to detect a difference from a mean value of 2.5 pg/mL to 1.1 times the within-person standard deviation with 80% power.

We assume that 25% of participants will have positive antigen at entry, we will have 62% power to detect a reduction to < 1% with positive antigen on the treatment arm.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) or paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Investigator (or designee) but will be identified by their unique PID.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. For paper CRFs: If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

16.2 Data Management Procedures

The data will be entered into a validated database (REDCap). The Data Management group will be responsible for data processing, in accordance with procedural documentation.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the study team for resolution. The study database will be updated in accordance with the resolved queries.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data is cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Investigator is required to maintain study records and, therefore, the Investigator should be contacted prior to removing study records for any reason.

As outlined above, after completion of the primary analyses, samples may be stored indefinitely via the LIINC biorepository for use on other COVID-related pathogenesis studies (as addressed in the consent form), at the discretion of the PI.

16.6 Monitoring

Monitoring visits will be conducted by representatives of the Investigator according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the safety medical committee (SMC), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject PID will identify all study subjects on CRFs and other documentation. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Investigator or their designee. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days. Protocol amendments will be sent to Shionogi in addition to the IRB/IEC and FDA as required.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs approval statement will be transmitted by the Investigator to Shionogi. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The CanRB/CanEC must be cannformed of re"cansio's to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part

56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization for submission to the IRB/IEC. The consent form generated by the Investigator must be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will store an IRB/IEC-approved copy of the Informed Consent Form for the study file.

A properly executed, written, cannformed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form will be offered to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Investigator and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying Shionogi, except when to protect the safety, rights or welfare of subjects.
25. Personally conduct or supervise the study (or investigation).
26. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
27. Report to Shionogi or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
28. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
29. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with Shionogi (or designee).
30. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
31. Promptly report to the IRB and Shionogi (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
32. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
33. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1. SCHEDULE OF EVENTS

Day	-28 to -7	-21 to 0	0-4	1	4	10	30	60
Visit Name	**Screen[1]**	**Baseline[1]**	**Intervention[2]**	**Phone FU D1**	**Phone FU D4**	**D10**	**D30**	**Phone FU EOS**
Visit Window	**N/A**	**N/A**	**N/A**	**+1**	**+/-1**	**+3/-3**	**+/-7**	**+/-14**
Screening Procedures								
Informed Consent	x							
Eligibility Review	x							
Screening Labs	x							
Pregnancy Testing	x							
Demographics	x							
Medical History	x							
COVID-19 History	x							
Concomitant Medications	x	x	x	x	x	x	x	x
Study Product Administration								
Randomization			x					
Ensitrelvir vs Placebo			x					
Clinical Assessments								
Vital Signs	x	x	x			x	x	
Physical Assessment	x	PRN	PRN			PRN	PRN	
Symptom Assessment	x	x	x		x[3]	x	x	x[3]
Quality of Life	x	x	x		x[3]	x	x	x[3]
PROMIS-29	x	x				x	x	
PGIC Scale					x	x	x	x
Duke Activity Status Index	x	x				x	x	
Dysautonomia Assessment		x				x	x	
ECOG (LIINC)		x				x		
DSQ-PEM		x			x[3]	x	x	

Day	**-28 to -7**	**-21 to 0**	**0-4**	**1**	**4**	**10**	**30**	**60**
Visit Name	**Screen[1]**	**Baseline[1]**	**Intervention[2]**	**Phone FU D1**	**Phone FU D4**	**D10**	**D30**	**Phone FU EOS**
Visit Window	**N/A**	**N/A**	**N/A**	**+1**	**+/-1**	**+3/-3**	**+/-7**	**+/-14**
WHO-DAS		x				x	x	
Neurocognitive assessment		x				x		
6MWT		x				x		
Safety Outcomes								
AE Review	x	x	x	x[3]	x[3]	x	x	x
Safety Labs			x			x		
Real-time laboratory tests								
Pregnancy testing	x		x[4]				x	
Complete metabolic panel	x		x			x		
CBC/diff	x		x			x		
Hep B surface antigen *Within 6 months of Screen*	x							
Hep C antibody *Within 6 months of Screen*	x							
Hep C RNA (if Ab positive) *Within 6 months of Screen*	x							
Plasma HIV Ab/antigen combination testing *Within 2 months of Screen*	x							
C-reactive protein		x	x[5]			x	x	
SARS-CoV-2 PCR or Ag test	x	PRN	PRN			PRN	PRN	
Post-hoc laboratory tests								
Biospecimen storage	x	x	X[5]			x	x	
Markers of viral persistence	x	x	x[5]			x	x	
Markers of inflammation		x	x[5]			x	x	

[1] For participants who received placebo and are opting to participate in study drug rollover, the repeat screening and baseline visits may be combined at the investigators discretion but the repeat baseline visit must be repeated as with the initial schedule of events.

[2] The baseline visit and intervention visit may be combined to minimize participant burden.

[3] Assessments at the D1 Phone FU visit will focus on adverse events since taking the medication. Assessments at the D4 Phone FU visit will focus on the preceding 2 days (rather than the preceding month) to assess AEs and the potential impact of treatment.

[4] For persons of childbearing potential, a negative pregnancy test must be confirmed prior to the participant being dispensed the medication vs placebo.

[5] If the intervention visit is on the same day as the baseline visit, blood is collected for biospecimen storage will only be collected once.

- **APPENDIX 2. CONTRACEPTION GUIDANCE**

CONTRACEPTIVES[a] ALLOWED DURING THE STUDY INCLUDE:
Highly Effective Methods[b] That Have Low User Dependency Failure rate of < 1% per year when used consistently and correctly
• Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c] PLUS an additional barrier method • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS)[c] • Bilateral tubal occlusion • Vasectomized partner (*Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.)* • Combined (estrogen- and progestogen-containing) hormonal contraception[c] associated with inhibition of ovulation (eg, oral, intravaginal, transdermal, injectable) PLUS an additional barrier method • Progestogen-only hormone contraceptive[c] associated with inhibition of ovulation: oral, injectable PLUS an additional barrier method
Highly Effective Methods[b] That Are User Dependent Failure rate of < 1% per year when used consistently and correctly
• Sexual abstinence *(Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)*
a Contraceptive use by men or women should be consistent with regulations regarding the methods of contraception for those participating in clinical studies. B Failure rate of < 1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly. C S-217622 may lead to an increase or decrease of hormonal contraception levels. Therefore, hormonal contraception must not be used alone and must be combined with other barrier method. Note: Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method are not acceptable methods of contraception. Male condom and female condom should not be used together (due to risk of failure with friction).

- **APPENDIX 3. PROHIBITED MEDICATION**

Use of the following therapies will be prohibited from the time of informed consent through 28 days after the first dose of study intervention:

- Sensitive CYP3A substrates with narrow therapeutic index. S-217622 is considered to be a strong inhibitor of CYP3A4. (Note: remdesivir and combined oral contraceptives are permitted). Examples of sensitive CYP3A substrates with narrow therapeutic index include the following medications: alfentanil, cyclosporine, sirolimus, tacrolimus and terfenadine

Use of the following therapies is prohibited from the time of informed consent through 10 days after the last dose of study intervention. However, even if a prohibited concomitant therapy is used, the participant should continue the study procedures according to the scheduled of event (Appendix 1) to the extent possible.

- Hydroxychloroquine, and ivermectin are prohibited due to DDIs with S-217622. Hydroxychloroquine and ivermectin are both CYP3A substrates and thus may also be substantially impacted by S-217622 with an increase in drug levels.
- Paxlovid (nirmatrelvir/ritonavir): Potential clinically significant interaction is likely expected when administered with S-217622. Coadministration of itraconazole, strong CYP3A4 inhibitor (200 mg once daily 8 doses), and nirmatrelvir/ritonavir (300/100 mg twice daily 5 doses) increased nirmatrelvir AUC and C_{max} by 39% and 19%, respectively.
- Corticosteroids administered via any route (including intranasal, inhaled, oral, intra-articular but excluding topical). However, prednisolone is permitted based on a lack of significant interaction in a clinical DDI study. Intranasal or inhaled fluticasone is permitted because the DDI potential by CYP3A inhibitor would be low. Furthermore, intranasal or inhaled beclomethasone is allowed based on demonstration of the lack of a significant interaction with darunavir/ritonavir, and dexamethasone can be administered the day after the last dose of study intervention administration based on the results of clinical DDI study with dexamethasone.
- CYP3A substrates except for sensitive CYP3A substrates with narrow therapeutic index. S-217622 is considered to be a strong inhibitor of CYP3A4. (Note: remdesivir and combined oral contraceptives are permitted). Examples of prohibited CYP3A substrates include the following medications: alprazolam, anamorelin, aprepitant, atorvastatin, avanafil, azelnidipine, blonanserin, budesonide, buspirone, chloroquine, colchicine, conivaptan, darifenacin, darunavir, dasatinib, dihydroergotamine, dronedarone, ebastine, eletriptan, eliglustat, eplerenone, ergometrine, ergotamine, everolimus, felodipine, finerenone, ibrutinib, indinavir, ivabradine, lomitapide, lovastatin, lurasidone, maraviroc, methylergometrine, midazolam, naloxegol, nisoldipine, pimozide, quetiapine, quinidine, rilpivirine, riociguat, rivaroxaban, saquinavir, sildenafil, simvastatin, suvorexant, tadalafil, ticagrelor, tipranavir, triazolam, tolvaptan, vardenafil, and venetoclax. For additional CYP3A

substrates, see the Liverpool DDI checker (https://www.covid19-druginteractions.org/checker).

Use of the following therapies is prohibited from the time of informed consent through the day after the last study intervention.

- Strong CYP3A inducers. Examples include apalutamide, carbamezpine, enzalutamide, mitotane, phenytoin, phosphenytoin, rifampin and St. John's wort. For additional strong CYP3A inducers, see the Liverpool DDI checker (https://www.covid19-druginteractions.org/checker).
- Methotrexate as organic anion transporter 3 (OAT3) substrate with a narrow therapeutic index.
- Digoxin and dabigatran as P-gp substrates with a narrow therapeutic index.
- High dose of rosuvastatin (20 to 40 mg, as it is considered BCRP substrate and S-217622 may influence the treatment by rosuvastatin when high dose of rosuvastatin is administered).

APPENDIX 4. OPEN LABEL EXTENSION (OLE) SCHEDULE OF EVENTS

Participants who received the placebo and opt to participate in the open label extension will follow the SOE outlined below. This SOE will serve as the final resource for guiding OLE activities.

Day	**-28 to -7**	**-21 to 0**	**0-4**	**1**	**4**	**10**	**30**
Visit Name	**Eligibility Confirmation**	**Baseline**	**Intervention[1]**	**Phone FU D1**	**Phone FU D4**	**D10**	**D30**
Visit Window	**N/A**	**N/A**	**N/A**	**+1**	**+/-1**	**+3/-3**	**+/-7**
Screening Procedures							
Informed Consent	x						
Eligibility Review	x						
Screening Labs	x						
Pregnancy Testing	x						
Medical History	x						
COVID-19 History	x						
Concomitant Medications	x	x	x	x	x	x	x
Study Product Administration							
Ensitrelvir			x				
Clinical Assessments							
Vital Signs	x	x	x			x	x
Physical Assessment	PRN	PRN	PRN			PRN	PRN
Symptom Assessment	x	x	x		x[2]	x	x
Quality of Life	x	x	x		x[2]	x	x
PROMIS-29	x	x				x	x
PGIC Scale					x	x	x
Safety Outcomes							
AE Review	x	x	x	x[2]	x[2]	x	x
Safety Labs			x			x	

Real-time laboratory tests							
Pregnancy testing	x		x[3]				x
Complete metabolic panel	x		x			x	
CBC/diff	x		x			x	
Hep B surface antigen *Only if new risk factors*	x						
Hep C antibody *Only if new risk factors*	x						
Hep C RNA (if Ab positive) *Only if new risk factors*	x						
Plasma HIV Ab/antigen combination testing *Only if new risk factors*	x						
C-reactive protein		x	x[4]			x	x
SARS-CoV-2 PCR or Ag test	x	PRN	PRN			PRN	PRN
Post-hoc laboratory tests							
Biospecimen storage	x	x	x[4]			x	x
Markers of viral persistence	x	x	x[4]			x	x
Markers of inflammation		x	x[4]			x	x

[1] The baseline visit and intervention visit may be combined to minimize participant burden.

[2] Assessments at the D1 Phone FU visit will focus on adverse events since taking the medication. Assessments at the D4 Phone FU visit will focus on the preceding 2 days (rather than the preceding month) to assess AEs and the potential impact of treatment.

[3] For persons of childbearing potential, a negative pregnancy test must be confirmed prior to the participant being dispensed the medication

[4] If the intervention visit is on the same day as the baseline visit, blood is collected for biospecimen storage will only be collected once.

APPENDIX 5. PROTOCOL AMENDMENTS

Date	Version	Section	Changes
2023-JUN-22	1.1	N/A	N/A
2023-AUG-15	1.2	Exclusion criteria	Exclusion criteria was updated to allow experimental PET imaging within 14 days of Day 0 rather than 28 days for all other interventional studies.
2023-AUG-15	1.2	Safety Monitoring	The independent medical monitor has been replaced by a safety monitoring committee. The monitoring schedule has not been changed.
2023-NOV-27	1.4	All	Corrected references (previously two separate reference lists now consolidated into one)
2023-NOV-27	1.4	6.2	1 – Removed mention of sex/gender from inclusion criteria – all adults regardless of sexual or gender identity are eligible and the unnecessary detail created confusion 3 – Simplified item 3 which was previously redundant 4 – Clarified at least two symptoms or at least one severe symptom. Added that at least 90 days have elapsed since the most recent SARS-CoV-2 infection 7 – Corrected criterion 7 to indicate “In otherwise stable health prior to Screen” rather than “D0” since eligibility for the protocol must be determined prior to D0. 8 – Clarified that study entry is defined as D0 9 – Added additional detail about the timing requirements of contraception to make internally consistent.
2023-NOV-27	1.4	6.3	2 – Clarified that that no SARS-CoV-2 antiviral should have been administered within 60 days prior to D0 (not just 60 days prior to D0) 4 – Clarified cardiovascular disease within 90 days should be excluded (not ever) – this is consistent with other UCSF-based Long COVID study protocols; also clarified

			that those with current heart failure or pulmonary hypertension are excluded but those with resolved heart failure are eligible. 11 – Indicated that these laboratory tests occur at screening. Corrected hemoglobin units. 12 – Added ESRD (FDA recommendation) 14 – Added severe hepatic impairment (FDA recommendation) 15 – Clarified that any HIV infection is exclusionary. Added clarification about steroid therapy. 16, 17 – specified that those with a history of ME/CFS or dysautonomia that is not worsened since SARS-CoV-2 infection would be ineligible 19 – revised to create consistency with Exclusion Criterion #1 for monoclonal antibodies or equivalent products, and to provide additional detail about timing since investigational PET tracers Adjusted multiple other criteria to anchor on planned D0, since eligibility needs to be determined prior to D0.
2023-NOV-27	1.4	9.1.4	Clarified that a physician, nurse, or PA may complete the physical assessments.
2023-NOV-27	1.4	9.1.5	Indicated that vital signs in various positions may be obtained.
2023-NOV-27	1.4	9.1.6	Indicated details about Long COVID assessments versus Other Symptom Assessment (AE Review) Added detail about PGIC Added details of ECOG Clarified that a neurocognitive assessment other than NIH Toolbox might be used. Removed Post-COVID function scale, which has proven to be insensitive in other studies by our group and burdensome to participants Clarified that a dysautonomia assessment other than COMPASS-31 may be performed.

			Added detail about DSQ-PEM. Added detail about WHO-DAS.
2023-NOV-27	1.4	8.4.2	Added detail about medication dispensing and logging.
2023-NOV-27	1.4	10.1	Referred to the Schedule of Events for screening procedures. Added note about rescreening.
2023-NOV-27	1.4	10.2	Added note that the D0 visit may be combined with the baseline visit. Added additional details about visit window.
2023-NOV-27	1.4	10.3	Added additional safety check prior to D0 to ensure no new changes to health have occurred since screen. Added detail that negative pregnancy test must be confirmed prior to dispensing study drug.
2023-NOV-27	1.4	9.3.3	Added additional detail about SARS-CoV-2 genotyping
2023-NOV-27	1.4	10.4	Added additional phone follow-up.
2023-NOV-27	1.4	10.5	Combined D7 and D15 visits into a single D10 visit to (1) minimize participant burden with one fewer in person visit (2) improve the consistency in timing of measurement for the primary outcomes of the study.
2023-NOV-27	1.4	10.8	Corrected discontinuation visits to sync with the new SOE.
2023-NOV-27	1.4	12.3	Added details about liver toxicity monitoring and overdose monitoring
2023-NOV-27	1.4	14	Edited SMC meeting frequency to every 4 months to sync with our other Long COVID

			clinical trials. Indicated that the study would be paused for grade 3 or 4 AEs or SAEs in any participant (FDA recommendation) Clarified rules around study pause to indicate that scheduled visits at which study product is not dispensed (screening, baseline, and follow up visits) may still occur but no new study product may be dispensed.
2023-NOV-27	1.4	SOE	Amended schedule of events to reflect protocol changes (extra phone visit, one fewer in person visit) Added PGIC scale to be consistent with other Long COVID trials Added ECOG (LIINC Form) Removed COMPASS-31 due to licensing issues; will now use the orthostatic hypotension questionnaire which does not have licensing issues Added DSQ-PEM, an assessment of post-exertional malaise Added WHO-DAS as a disability scale, at recommendation of patient groups Removed mention of NIH Toolbox; will use CNS-Vital Signs like other UCSF-based Long COVID trials Corrected safety labs from baseline to intervention visit; this was already indicated in the “real-time laboratory tests” below and does not represent a change Removed ESR, fibrinogen, and D-dimer as real-time laboratory tests. These have not been useful in other Long COVID studies and may be performed post-hoc. Added note that baseline and intervention visit may be combined to minimize participant burden.
2024-FEB-21	1.5	2.3.1	Updated evaluation of anemia monitoring to match with SOE.
2024-FEB-21	1.5	5.1	1 – Corrected the endpoint visit for Promis-29 to match with SOE 2 – Removed mention of NIH Toolbox; will use CNS-Vital Signs like other UCSF-based Long COVID trials

			3 – Corrected the endpoint visits for neurocognitive assessment, DASI, dysautonomia assessment and 6MWT to match with SOE 4 – Corrected Day 15 to Day 10
2024-FEB-21	1.5	12.3	Corrected end of study visit from Day 360 to Day 60
2024-FEB-26	1.5	10.3	Removed language requiring first dose of study intervention to be taken at home
2024-OCT-15	1.6	Inclusion Criteria	Updated effective contraception methods requirement to begin 21 days prior to the start of study intervention
2024-OCT-15	1.6	9.2.1	Removed mention of ESR to match SOE update from version 1.4
2024-NOV-05	1.6	Appendix 4	Added Open Label Extension Schedule of Events for after unblinding rollover dosing
2024-NOV-05	1.6	Appendix 5	Appendix 4 in version 1.5 “Tracked Changes” has been relabeled Appendix 5
2024-NOV-05	1.6	Entire Protocol	Updated Version Date to match most current date of changes, corrected formatting on table of contents

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

PLACEBO-CONTROLLED, RANDOMIZED TRIAL OF ENSITRELVIR (S-217622) FOR VIRAL PERSISTENCE AND INFLAMMATION IN PEOPLE EXPERIENCING LONG COVID (PREVAIL-LC)

NCT06161688

Statistical Analysis Plan Finalization Date: 02/21/2025

Statistical Analysis Plan (SAP)

Title: Placebo-Controlled, Randomized Trial of Ensitrelvir (S-217622) for Viral Persistence and Inflammation in People Experiencing Long COVID (PREVAIL-LC)

Primary Investigator: Timothy Henrich, MD

Co-Investigator: Michael Peluso, MD

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Version: 1.0

Version Date: February 2025

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Date: 2/21/2025

1. Introduction

The objective of this statistical analysis plan (SAP) is to describe the general analytic strategy and the statistical methods that will be used to analyze the data for “PlaceboControlled, Randomized Trial of Ensitrelvir (S-217622) for Viral Persistence and Inflammation in People Experiencing Long COVID (PREVAIL-LC).

2. Study Design

2.1. Randomization and Follow-Up

This is an exploratory, 1:1 randomized, double-blind, placebo-controlled study to assess the safety and efficacy of Ensitrelvir (S-217622) to treat Long COVID (LC). The study will enroll approximately 40 participants who meet the WHO LC criteria.

Participants will be enrolled at a single center and randomized 1:1 to receive 5 days of Ensitrelvir or placebo. Evaluations will take place at baseline and at timepoints up to 2 months post-administration.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria (see study protocol) will be entered into the study.

The following treatment regimens will be used:

Route	**Planned Dose**	**Dose**
Oral	Ensitrelvir	375 mg on day 1, followed by 125 mg daily for 4 additional days
	Placebo	NA

The total duration of subject participation will be approximately 3 months. In-person study visits will occur at screening, baseline, intervention, and days 10 and 30 (D10 and D30, respectively) following administration. Additional interviews will be conducted by phone on days 1, 4, and 60 (D1, D4 and D60).

2.2. Study Endpoints

Efficacy Endpoints

- Change in PROMIS-29 Physical and Mental health score in those with symptoms at Baseline and at D10 and D30.
- Change in EuroQoL Quality of Life between Baseline and at D10, D30, and D60.
- Change in other assessments (neurocognitive assessment (i.e. ECog, CNS-VS) and 6MWT performance) between Baseline and at D10.
- Change in other assessments (DASI, dysautonomia assessment) between Baseline and at D10 and D30.
- Percentage of participants with no detection of SARS-CoV-2 plasma remnants (i.e., viral detection by reverse transcriptase-polymerase chain reaction (RT-PCR) and Spike protein fragments) compared to baseline at D10 and D30 post-ensitrelvir

administration. ● Proportion with reduction in inflammatory markers (e.g. CRP) at Baseline and at D10 and D30.

2.3. Safety Endpoints

The National Institutes of Health Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 (July 2017) will be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant.

Criteria for relatedness of adverse events is given Sec 11.1.2 of the study protocol. Criteria for SAE are given in Sec 11.2 of the protocol.

- Safety endpoints include number of participants with adverse events (AE)s (Treatment Emergent Adverse Events (TEAE)s, Serious Adverse Events (SAE)s, and Adverse Events of Special Interest (AESI). The primary outcome will be the number of individuals in each group experiencing a severe (Grade 3 or greater) AE thought to be possibly, probably, or definitely related to study treatment throughout the study duration.

2.4. Sample Size and Study Power

This is an exploratory study, and the primary results are meant to be descriptive. We use Ensitrelvir as a probe to better understand LC biology and determine whether a signal is present to warrant further study.

The study will enroll 40 participants who meet the WHO LC criteria. Participants will be enrolled at a single center and randomized 1:1 to receive Ensitrelvir or placebo.

For PROMIS-29 Physical Health Score, assuming a standard deviation (SD) of the within-person difference of 10, then the planned sample size has 80% power to detect a difference of 11.2 on a two-sided 0.05 level test.

For biomarkers, such as CRP, we can detect a difference from a mean value of 2.5 pg/mL to 1.1 times the within-person standard deviation with 80% power.

We assume that 25% of participants will have positive antigen at entry, we will have 62% power to detect a reduction to < 1% with positive antigen on the treatment arm.

3. Analysis Principles

The following principles apply for the comparisons of the participants randomized to Ensitrelvir (S-217622) (“active group”) versus the participants randomized to placebo (“control group”).

3.1. Analysis populations

Comparisons for *safety and efficacy outcomes* will be *by modified intention-to- treat (mITT).* The modified intention-to-treat analysis is restricted to participants who received at least one dose of blinded Ensitrelvir/placebo; participants who did not receive *any* Ensitrelvir//placebo are excluded from the mITT population.

Sensitivity analyses by *intention-to-treat* (ITT) will be carried out for the primary efficacy outcome and key secondary outcomes. The ITT population is all participants who were randomized during the study and assigns them to the group to which they were randomized.

The *per protocol population* (PP) is restricted to participants who received any of the blinded Ensitrelvir/Placebo. It excludes participants who received a non-study COVIDantiviral therapy during the trial period (prior to Day 30).

The safety population will be identical to the mITT population.

3.2. Descriptive statistics

Descriptive statistics will be reported overall and by randomization group. For categorical outcomes, the number and percent in each category will be reported; percentages will be of non-missing values, if data are not complete. Continuous variables will be summarized by median (interquartile range [IQR]) and/or mean (SD). Continuous variables may be categorized (e.g., age may be broken into categories to investigate the distribution across age groups).

3.3. Binary outcomes

For binary outcomes, probabilities will be compared between the investigational agent and its control group using Fisher's exact tests or logistic regression. Proportions will be accompanied by Agresti Coull 95% confidence intervals[1]. Relative risks will be calculated with 2-sided 95% confidence intervals. Risk difference (RD) with 2-sided 95% confidence intervals (CI) will also be compared between the groups. If the numbers in the denominators are small, our confidence intervals will use the pseudo-observation approach of Agresti and Caffo.[2]

For longitudinally measured binary outcomes, the treatment effect, in relative risks, through follow-up will be estimated with 95% confidence intervals using generalized

estimating equations (GEE) with a Poisson family and a log link function; the treatment effect is estimated via the interaction between the indicator for randomization group and the binary indicator for follow-up (versus baseline) visits. Here, we may also employ the pseudo-observation approach to calculating the confidence intervals if the outcomes are rare. [2] The treatment effect, in risk differences, with 95% confidence intervals will be estimated by margining the models and calculating 95% confidence intervals by the chain rule.

3.4. Ordered categorical outcomes

Ordinal variables will be compared between randomization groups using WilcoxonMann-Whitney. We may summarize effects quantifying the treatment effects using the proportional odds model with the summary OR of being in a better category in the group compared to the placebo group, adjusted for baseline category, will be estimated with a 2-sided 95% CI The validity of the proportional odds assumption will be assessed by testing the log ORs (for the treatment effect) across the dichotomized cumulative ordered categories in the corresponding ordered logistic regression model (partial proportional odds model, test for “unequal slopes”)[3]. For longitudinal measured ordinal variables, the treatment effect through follow-up will be estimated with 95% confidence intervals using generalized estimating equations (GEE) with a logistic link function; the treatment effect is estimated via the interaction between the indicator for randomization group and the binary indicator for follow-up (versus baseline) visits.

3.5. Continuous outcomes

Continuous outcomes will be compared between randomization groups using ANCOVA models for comparing means, if the ANCOVA model assumptions hold. If the distributions of the continuous outcomes are skewed, outcomes may be transformed or compared between randomization groups using rank-based methods, such as the Wilcoxon test, or quantile (median) regression. For example, biomarker levels often require log-transformation to meet model assumptions for ANCOVA analyses.

Comparisons between randomization groups for a continuous outcome will be adjusted for baseline values of the outcome, for the purpose of variance reduction, unless there are concerns over model stability with such an adjustment.

To estimate the treatment effect for *longitudinally measured continuous outcomes*, including those measured at baseline and just once post-baseline. We will use linear mixed effects models (in case of Gaussian responses) with an indicator for randomization group, visit and visit by group interaction. The model will include an unstructured covariance structure. Models will be adjusted for the baseline values of the

outcome variable. As needed, continuous outcomes may be transformed to fulfil the model assumptions (e.g., log-transformation). The estimand will be defined as net "change from baseline" (difference at follow-up visit minus baseline value). The treatment effect through follow-up will then be estimated with 95% confidence intervals

3.6. Visit Windows, Interim Visit and Mistimed Visits

Visits will be categorized using the schema below by the Clinical Research Coordinators (CRCs) of the study. Not every visit will have taken place on the Target Day, and acceptable visit windows (in days) are shown below.

Visit	Screen	Baseline	Intervention	Phone FU D1	Phone FU D4	D10	D30	Phone FU D60
Target Day	**-28**	**-7**	**0**	**1**	**4**	**10**	**30**	**60**
Visit Window (days)	**-28 to 7**	**-21 to 0**	**0-4**	**+1**	**+/-1**	**+/-3**	**+/-3**	**+/-14**

We will also group visits by defining an extended window around visit target dates. The windows will be the dates which are mid-way between the target visit dates. If there is more than 1 visit in an extended window, then the closer would to the target date will be used. Hence, a visit out of the desired window defined in the table is not excluded. We will perform a sensitivity analysis which will include only visits which fall in the visit window.

3.7. Missing Values

Our primary analysis will use the available visits without adjustment for missing values.

We will conduct sensitivity analyses will use chained equations to multiply impute missing outcomes data. To this end, we will use the available panel of the measures, demographics and values of other available similar measures.

3.8. Intercurrent Events

Our analysis may be affected by SARS-CoV-2 reinfections during the study period. Our primary analysis will include all visits during the study period. We will perform sensitivity analyses which exclude any visits after a documented or suspected on study reinfections.

3.9. Subgroup analyses

We will perform subgroups analyses by participant sex and antigen detection at baseline. A subgroup effect will be tested by a two-sided 0.05 level test of interaction.

4. Efficacy Analyses

The primary efficacy analyses will be conducted in the mITT population. Analysis in the per protocol population will be considered a sensitivity analysis.

4.1. Primary Efficacy Outcome

The primary efficacy outcome is the change in PROMIS-29 Physical Health score from Baseline to D10. The baseline value for the analysis will be the mean of screening and baseline values. This will be analyzed from the mITT population using a linear regression model for follow-up PROMIS-29 Physical Health score with terms for the baseline value, randomized treatment – an analysis of covariance model (ANCOVA).

The significance of the treatment comparison will be given by a two-sided 0.05 level test for the randomized treatment term.

The mean PROMIS-29 Physical Health score to D10 can be calculated by margining the ANCOVA model above.[4]

Note, these estimates will differ from both taking the mean PROMIS-29 at the baseline and D10 timepoints. However, the ANCOVA estimate is more efficient.

4.2. Secondary Efficacy Analyses

Secondary efficacy outcome will be analyzed from the mITT population. There will be no formal adjustment for multiple testing in analyzing the secondary endpoints. Secondary outcomes are listed in 4.2.1, 4.2.2 and 4.2.3.

4.2.1 Questionnaires

- Change in PROMIS-29 Physical Health summary score from Baseline to D30.
    - The primary estimand for this outcome is the difference in mean PROMIS29 Physical Health summary score from baseline. The baseline value for the analysis will be the mean of screening and baseline values.
    - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in PROMIS-29 Mental Health summary score from Baseline to D10.
    - The primary estimand for this outcome is the difference in mean PROMIS29 Mental Health summary score from baseline.
    - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in PROMIS-29 Mental Health summary score from Baseline to D30.
    - The primary estimand for this outcome is the difference in mean PROMIS29 Mental Health summary score from baseline.
    - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- LIINC Questionnaire – Quality of Life by Visual Analogue Scale (VAS) from Baseline to D10.
    - The primary estimand for this outcome is the difference in mean VAS score from baseline. The baseline value for the analysis will be the mean of screening and baseline values. ○ This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Quality of Life Using the 5-Item EuroQol EQ-5D-5L index value score from Baseline to D10. ○ The primary estimand for this outcome is the difference in mean the EQ5D-5L index value from Baseline to D10. The baseline value for the analysis will be the mean of screening and baseline values.
    - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in Duke Activity Status Index (DASI) from Baseline to D10.
    - The primary estimand for this outcome is the difference in mean DASI total score from baseline. The baseline value for the analysis will be the mean of screening and baseline values. ○ This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in the World Health Organization Disability Assessment Schedule 2.0 (WHO-DAS 2.0) questionnaire from Baseline to D10.

  - The primary estimand for this outcome is the difference in mean WHODAS 2.0 score from baseline.
  - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Patient Global Impression of Change (PGIC) at D10.
  - The primary estimand for this outcome is the relative odds of better change on the PGIC at D10 between the arms. ○ This ordinal outcome will be analyzed as described in Section 3.4.

- Change in the Everyday Cognition Form (ECog-41) instrument from Baseline to D10.
  - The primary estimand for this outcome is the difference in mean ECog-41 score from baseline. ○ This continuous outcome will be analyzed as described in Section 3.5, i.e.
    using a mixed effect model for repeated measurements.

- Change in Orthostatic Intolerance Questionnaire (OHQ) composite score from Baseline to D10. ○ The primary estimand for this outcome is the difference in mean OHQ composite score from baseline.
  - This continuous outcome will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

4.2.2 Performance Measures

- Change in 6 Minute Walking Test (6MWT) from Baseline to D10.
  - The primary estimand for this outcome is the difference in mean 6MWT change from baseline. ○ This continuous outcome will be analyzed as described in Section 3.5 , i.e.
    using a mixed effect model for repeated measurements.

- Change in Active Stand Test Result from Baseline to D10.
  - The primary estimand for this outcome is the relative proportion with postural orthostatic tachycardia syndrome by the stand test. ○ This binary outcome will be analyzed as described in Section 3.3, i.e.
    using a GEE model.

- Change in Neurocognition Index (NCI) standard score from the CNS-VS from Baseline to D10.

- The primary estimand for this outcome is the difference in mean change in the NCI standard score from the CNS-VS from baseline.
- This continuous outcome will be analyzed as described in Section 3.5 , i.e. using a mixed effect model for repeated measurements.

4.3.Exploratory Efficacy Analyses

4.3.1 Questionnaires

- Change in the LIINC Questionnaire – Symptom Assessment between Baseline and D4, D10, D30 and D60.
  - The primary estimand for these continuous outcomes are the difference from baseline for each respective score.
  - These continuous outcomes will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in DASI, OHQ, WHODAS (2.0) from Baseline to D30.
  - The primary estimand for these continuous outcomes are the difference from baseline for each outcome.
  - These continuous outcomes will be analyzed as described in Section 3.5, i.e. using a mixed effect model for repeated measurements.

- Change in DePaul Symptom Questionnaire (DSQ) post-exertional malaise (PEM) from Baseline D4, D10, D30.
  - The primary estimand for this outcome is the relative proportion with PEM.
  - This binary outcome will be analyzed as described in Section 3.3, i.e. using a GEE model.

 Patient Global Impression of Change (PGIC) at D4, D30, D60.
  - The primary estimand for this outcome is the relative odds of better change on the PGIC between the arms.
  - This ordinal outcome will be analyzed as described in Section 3.4.

4.3.2 Performance Measures

 Change in Active Stand Test result from Baseline to D30.

○ The primary estimand for this outcome is the relative proportion with postural orthostatic tachycardia syndrome by the stand test. ○ This binary outcome will be analyzed as described in Section 3.3, i.e. using a GEE model.

4.3.3 Laboratory Measures

- Change from Baseline to D10, D30 in C-reactive protein (CRP)
  - ○ The primary estimand for these outcomes is the difference in mean change from baseline.
  - ○ These continuous outcomes will be analyzed as described in Section 3.5 , i.e. using a mixed effect model for repeated measurements.

- Percentage of participants with no detection of SARS-CoV-2 plasma remnants (i.e., viral detection by reverse transcriptase-polymerase chain reaction (RT-PCR) and Spike protein fragments) compared to Baseline through D10 and D30.

5. Safety Analysis

The primary outcome will be the number of individuals in each group experiencing a severe (Grade 3 or greater) Adverse Event (AE) attributed to be possibly, probably, or definitely related to study treatment will be compared between the arms. The number and proportion of participants treatment-emergent adverse events (TEA), serious adverse events (SEA) and Adverse Events of Special Interest (AESI) will also be summarized across treatment groups in the mITT population. Differences will be tested by a 0.05 level two-sided Fisher's exact test. Agresti and Coull confidence intervals will be calculated for proportions.[1]

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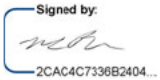
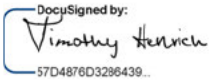
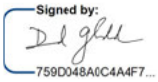
Dr. Michael Peluso [redacted] Assistant Professor University of California, San Francisco Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Not Offered via Docusign	Signed by:  2CAC4C7336B2404... Signature Adoption: Drawn on Device [redacted]	Sent: 2/20/2025 6:54:46 PM Viewed: 2/20/2025 6:54:51 PM Signed: 2/20/2025 6:55:01 PM
Timothy Henrich [redacted] Professor of Medicine University of California, San Francisco Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Not Offered via Docusign	DocuSigned by:  57D4876D3286439... Signature Adoption: Pre-selected Style [redacted]	Sent: 2/20/2025 6:55:03 PM Resent: 2/21/2025 10:08:46 AM Viewed: 2/21/2025 12:08:48 PM Signed: 2/21/2025 12:08:52 PM Sent: 2/21/2025 12:08:53 PM Viewed: 2/21/2025 12:10:56 PM Signed: 2/21/2025 12:12:02 PM
David Glidden [redacted] Professor University of California, San Francisco Security Level: Email, Account Authentication (Optional) **Electronic Record and Signature Disclosure:** Not Offered via Docusign	Signed by:  759D048A0C4A4F7... Signature Adoption: Uploaded Signature Image [redacted]	

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
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Certified Delivered	Security Checked	2/21/2025 12:10:56 PM
Signing Complete	Security Checked	2/21/2025 12:12:02 PM
Completed	Security Checked	2/21/2025 12:12:02 PM

Payment Events	Status	Timestamps