Clinical Trial Protocol

REVIVE_TOGETHER

"A Multicenter, Adaptive, Randomized, Double-Blind, Placebo-Controlled, Prospective Study of Pharmacological Interventions in Participants with Clinical Features Consistent with Long COVID-19: The REVIVE-TOGETHER Protocol"

OFFICIAL STUDY TITLE : A Multicenter, Adaptive, Randomized, doublE-blinded, Placebo-controlled Study in Participants With Long COVID-19: The REVIVE Trial

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STATEMENT
This following document is an English translation of the original Portuguese study protocol. It is provided for public posting and informational purposes in ClinicalTrials.gov. The original Portuguese version remains the source document maintained by the sponsor/ investigator in accordance with applicable local regulatory, ethics, and institutional requirements in Brazil.

CONFIDENTIALITY NOTE
No research participant names or directly identifying personal information are included in this document.

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"A Multicenter, Adaptive, Randomized, Double-Blind, Placebo-Controlled, Prospective Study of Pharmacological Interventions in Participants with Clinical Features Consistent with Long COVID-19: The REVIVE-TOGETHER Protocol"

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GLOSSARY OF TERMS

Evaluation	A procedure used to generate the data needed for the study
Cohort	A group of newly enrolled participants treated with a specific dose and regimen (i.e., treatment group) at the same time
Control medication	Any medication (an active drug or an inactive drug, such as a placebo) that is used as a comparator for the drug being tested in the study
Drug dose	The dose of the drug administered to the participant (daily or weekly total, etc.).
Inclusion	The point/time of entry into the study for which informed consent must be obtained (i.e., before initiating any procedure described in the protocol)
Period	A part of the study that serves a specific purpose. Typical periods include: selection/recruitment, *washout* period, treatment, and follow-up
Investigational drug	The drug whose properties are being tested in the study; this definition is consistent with US CRF 21, Section 312.3, and is synonymous with "new investigational drug" or "experimental medicinal product."
Treatment under investigation	All drugs whose properties are being tested in the study, as well as their associated control treatments. This *includes* any placebo, any active control, and also approved drugs used outside their approved indications/doses or tested in a fixed combination. The investigational treatment generally *does not include* concomitant background therapies specified by the protocol when these are standard treatments for that indication
Drug number	A unique identifier on the label of each package of the study/investigational drug in studies dispensing the drug using an IRT system
Protocol	A written record of all procedures to be followed in a study that describes all administrative, documentation, analytical, and clinical processes to be used in the study.
Arm	A single component of a study that contains different objectives or populations within a single study. Common parts of a study include: a single-dose part and a multiple-dose part, or a part involving participants with established disease and those with newly diagnosed disease.

Period	A subdivision of a crossover study
Premature withdrawal of a participant/patient	The point at which a participant leaves the study before the planned completion of all study treatment administrations and/or assessments; at this point, all study treatment administrations are discontinued and no further assessments are planned, unless the participant is followed up regarding disease progression and/or survival
Randomization number	A unique identifier assigned to each randomized participant, corresponding to a specific treatment arm assignment
Study drug/treatment	Any single drug or combination of drugs administered to the patient as part of the required study procedures; includes the investigational drug, active treatment periods (*run-in*), or background therapy
Discontinuation of study/investigational treatment	The point/time at which the participant permanently discontinues use of the study/investigational treatment for any reason; may or may not also be the point/time of the patient's early withdrawal
Participant number	A number assigned to each patient included in the study
Variable	A measured value or an assessed response that is determined at a specific assessment and used in data analysis to evaluate the investigational drug in the study

PROTOCOL OVERVIEW

Protocol - Long COVID Chronic Fatigue

Title:	**"A multicenter, adaptive, randomized, double-blind, placebo-controlled, prospective study of pharmacological interventions in participants with a clinical presentation consistent with Long COVID-19: The REVIVE-TOGETHER Protocol"**
Abbreviated Title:	Long COVID Chronic Fatigue Syndrome
Investigational product:	Metformin, Fluvoxamine
Indication:	Long COVID with chronic fatigue syndrome
Phase:	PHASE III – New indication
Sponsor:	CARDRESEARCH - Cardiology Care and Research, LLC
Study code	LONG-COVID_REVIVE_TOGETHER
Principal Investigators:	Gilmar Reis, Leonarco Cançado Monteiro Savassi, Edward J Mills, Lehana Thabane, Gordon H Guyatt
Proposing institution:	Cardresearch - Cardiology Care and Research, LLC
Collaborating Researchers Scientists	Ed. J. Mills, PhD. Lehana Thabane, PhD Gordon H Guyatt, MD
Objectives:	The primary objective is to compare metformin, fluvoxamine, and placebo for the mean Fatigue Severity Scale (FSS) score at 60 days after randomization. The secondary objectives are: 1. To compare metformin, fluvoxamine, and placebo for the mean FFS score 30 days after randomization. 2. To compare metformin, fluvoxamine, and placebo for the mean FSS score 90 days after randomization. 3. To compare metformin, fluvoxamine, and placebo regarding the mean difference in change in health-related quality of life (EQ-5D-5L) at 60 days after randomization. 4. Compare metformin, fluvoxamine, and placebo for the odds ratio of all-cause mortality at 60 days after randomization. 5. Compare metformin, fluvoxamine, and placebo for the odds ratio of unexpected hospitalization from all causes at 60 days. 6. To assess the safety of metformin and fluvoxamine in this population of participants. 7. To assess the medium-term effects (90 and 180 days) of the investigational medical products on the chronic fatigue scale (FSS scale) and quality of life (EQ-5D-5L).
Design:	A multicenter, double-blind, adaptive, prospective, randomized, parallel-group, placebo-controlled study evaluating metformin and fluvoxamine with a 90-day follow-up after randomization.
Inclusion Criteria	Participants must meet all of the following criteria to be eligible for participation in this study:

	1. Age 18 years or older at the time of screening. 2. Willing and able to provide written informed consent, or have a legal representative who can provide informed consent (when approved locally and nationally). 3. A previous SARS-CoV-2 infection confirmed by the participant (e.g., reports having had a positive nucleic acid amplification test or a positive professional-use or self-test SARS-CoV-2 rapid antigen test). 4. Participants with a clinical presentation consistent with LONG COVID according to international definitions: (www.nice.org.uk/guidance/ng188 , https://www.who.int/publications/i/item/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1), and fatigue symptoms with an average score of at least 03 on the Fatigue Scale (FSS); 5. Have had the most recent episode of COVID-19 within 24 months of screening; 6. Not currently hospitalized or in need of hospitalization, or having been hospitalized in an intensive care unit during the COVID-19 episode. 7. Participants with the following vital signs: a. Heart rate between 55 and 100 bpm; b. Temperature below 38°C; c. Oxygen saturation ≥ 95%. 8. Participants who are capable of becoming pregnant or whose partners are capable of becoming pregnant must agree to use appropriate contraceptive methods during the study and for up to 90 days of follow-up. 9. Symptoms of fatigue cannot be attributed to another cause (in the researcher's opinion). 10. Willingness to follow all study procedures.
Exclusion criteria	Participants who meet any of the following criteria will not be eligible to participate in the study: 1. Known acute SARS-CoV-2 infection; 2. Inability to understand the content of the Informed Consent Form or to follow the study procedures; 3. Known prior diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome, unrelated to SARS-CoV-2 infection; 4. Known pre-existing dysautonomia, unrelated to SARS-CoV-2 infection; 5. Diabetes mellitus (exclusion criterion for the Metformin and Metformin Placebo arms); 6. Known history of creatinine clearance below 30 mL/min or on dialysis. 7. Known stroke within the 3 months prior to screening; 8. Known severe anemia, defined as < 8 g/dL; 9. Body Mass Index (BMI) > 40. 10. Known diagnosis of Lyme disease; 11. Any use of illicit drugs other than marijuana within 30 days prior to informed consent;

	12. Pregnant women or women of childbearing age who do not agree to use an effective method of contraception for 90 days from the date of signing this consent form; 13. Women who are breastfeeding; 14. Expected hospitalization for elective surgical procedures lasting more than 48 hours; 15. Contraindications for the METFORMIN arm a. Participants currently taking metformin; b. Participants with creatinine clearance < 30 ml/min, a history of KIDIGO IV, or currently undergoing dialysis. 16. Contraindications for the FLUVOXAMINE arm a. Use of serotonin reuptake inhibitors (donepezil, fluoxetine, escitalopram, paroxetine); 17. Current severe comorbid psychiatric disorder (e.g., clinical depression, anxiety, sleep disorder, eating disorder, substance abuse) that is uncontrolled and associated with significant symptoms or requiring the use of a medication contraindicated in this study; 18. Clinical history of moderate to severe hepatic impairment or hepatic cirrhosis with a Child-Pugh score of C or higher; 19. Clinical history of severe chronic lung disease with significant limitation of activities; 20. Inability of the participant to provide informed consent or adhere to the procedures proposed in the study; 21. Taking medications known to cause chronic fatigue as a side effect (except for participants with chronic cardiovascular disease who are on chronic beta-blocker therapy); 22. Known hypersensitivity and/or intolerance to fluvoxamine or metformin; 23. Any clinical condition that, in the investigator's opinion, may preclude participation in this study.
Primary and secondary outcomes:	The primary outcome is a reduction in the FSS score of at least one standard deviation 60 days after randomization. The secondary outcomes are: 1. The FFS score 30 days after randomization. 2. The FFS score 90 days after randomization. 3. HRQL, assessed using the EuroQol-5D-5L 60 days after randomization. 4. All-cause mortality up to 60 days after randomization. 5. Unexpected hospitalization from any cause up to 60 days after randomization. 6. Safety and tolerability of the investigational products 7. Adverse events experienced by participants during treatment
Procedures	**Screening procedures**

	Before any study-specific procedure, the participant will receive an explanation of all study procedures and must date and sign an informed consent form (ICF) approved by a Research Ethics Committee (REC). The screening visit will be conducted in person. The ICF may be provided in physical, paper form or via an electronic informed consent form, in accordance with the guidelines recommended by CONEP. Eligible participants will be identified during the clinical consultation. Participants identified as having a probable diagnosis of Long COVID will be invited to learn about the research project. If they express interest, they will be referred to a designated and trained research team member who will present the proposed research program, verify the criteria for diagnosing Long COVID, and, if the diagnosis is confirmed, present the Informed Consent Form (ICF), which will be provided in accordance with current regulatory standards for clinical research. Research procedures will only begin if participants who express interest in participating in the research program sign the TCLE, which is the first step in the research process. During the screening visit, participants will receive a unique participant number, which will be generated during the registration of the screening visit in the IWRS. Female participants are initially screened to identify those who meet the eligibility criteria. After obtaining informed consent, participants of childbearing potential will be asked to take a pregnancy test. Participants will also be asked to complete the FSS to determine if they have a moderate score to qualify for participation. **Screening Visit Procedures and Randomization** The screening visit and randomization procedures should be performed immediately after confirming eligibility criteria and obtaining consent to participate in the study and may be performed during the same visit. The following procedures will be performed: 1. Obtaining the TCLE; 2. EQ-5D-5L questionnaire; 3. FSS Scale (chronic fatigue) 4. Verification of inclusion/exclusion criteria; 5. Urinary pregnancy test for females of childbearing age; 6. Registration in the IWRS; 7. Demographic data; 8. Medical history; 9. Comorbidities and risk factors; 10. Concomitant medications; 11. Physical examination findings; 12. Vital signs (including temperature, heart rate, respiratory rate, and oxygen saturation in room air); 13. Details of your COVID-19 medical history; 14. Randomization and administration of the first dose of the investigational product/placebo.

15. Delivery of the investigational product/placebo and instructions regarding it.
16. Instructions regarding subsequent visits.

Investigational product
Participants will receive the investigational products and instructions on dosing, which must be taken as directed for a period of 60 days.

Follow-up procedures
Follow-up visits will take place on days 7, 30, 60, 90, and 180 (± 7 days) after randomization. The visits on days 7, 30, 90, and 180 should preferably be conducted via telephone, telemedicine, or social media. The visit on day 60 should preferably be conducted at the research center, but may also be conducted via telephone, telemedicine, or social media. Participants will be asked to complete the FSS and the EQ-5D-5L 30 days, 60 days, and 90 days after randomization. In addition, on days 30, 60, and 90, the local research team will verify the participant's status and clinical outcomes (all-cause mortality and unplanned hospitalization). The research team will collect data on adherence to the experimental product and the placebo. The research team will also document concomitant medications. Participants will be monitored for adverse events and laboratory abnormalities at each visit.

The above data will be collected directly from the participant, the participant's medical record, or the healthcare professional(s) responsible for the participant's care. If a participant is unable to return to the healthcare center for a follow-up visit, the research team may contact the participant and conduct the visit by telephone. Data may also be collected via valid methods of electronic communication.

Study Committee
The event adjudication committee is responsible for ensuring that the source documents supporting the study event/outcome are adequate and that the diagnosis of adverse events is correct and supported by supporting documentation. In the absence of such documentation, events will be reviewed in accordance with good research practices, and the information certified by the principal investigator at each center will be attached to the study file.

	The independent Data Monitoring Committee (DMC) has a predefined plan for statistical data analysis and research safety that was approved prior to the start of this clinical trial. The analyses follow good clinical practices, being conducted in a blinded manner with possible unblinding regarding the indicated study arms if the pre-specified criteria for such unblinding are met (see the committee's composition in the document attached to the regulatory file). The steering committee has been in place since the start of the clinical trial, and its purpose is to ensure the scientific integrity of the study, as well as to oversee operational aspects for the proper conduct of the research.
Sample Size	The sample size is 1,500 participants, with 500 participants per treatment arm under evaluation, including the placebo arm, and its operational characteristics were confirmed through closed-form, analytical calculations, and numerical methods. The operational characteristics for the head-to-head comparison of the treatment arm versus the placebo arm in reducing the mean fatigue score measured by the Fatigue Severity Scale (1 to 9) on day 60 are determined. For each head-to-head comparison against the placebo arm, the Type I error rate (alpha) is set at 0.025 (one-sided) using a Bayesian normal-normal model. For the planning of our study, we assumed a plausible standard deviation of 1.7, which was estimated from a recent study that evaluated the psychometric properties of the Fatigue Severity Scale in participants recovering from COVID-19[1] . With the proposed sample size of 1,500 participants (3 arms containing 500 participants each), the study will have adequate statistical power to detect a clinically important treatment effect. A difference of 0.45 has been suggested as a minimally important difference (MID) on the Fatigue Severity Scale in population groups of participants with multiple sclerosis and chronic fatigue[2] . Even with a 10% dropout rate, our estimated power to detect a difference of 0.45 is above 90%. Interim analyses are planned upon reaching 25%, 50%, and 75% of the study population. The unblinded statistician advising the DMC will make recommendations based on the continuous evaluation of overall data and epidemiological data on Long COVID from Brazil and the state of Minas Gerais. We are continuously monitoring the literature for new findings relevant to chronic fatigue and to participants with Long COVID, and this protocol will be updated as needed based on relevant information and submitted to regulatory authorities for ethical review.
Statistical Methods	**Overview** **Study design** A phase III adaptive, randomized clinical trial to evaluate the efficacy and safety of new treatments and existing medications (new therapeutic

indications) in participants with symptoms of chronic fatigue from Long COVID-19 following resolution of acute COVID-19 infection.

Internal pilot phase

Phase to assess the feasibility of the study and address any issues that may arise, aiming to refine the trial, with a focus on identifying unforeseen feasibility problems and resolving them to improve the overall success of the research. In particular, we will evaluate issues related to recruitment, informed consent, availability and administration of medications, data collection and recording, and the safety and tolerability of the proposed interventions. There will be no analysis of clinical results at the end of this phase—since participants will be transferred to the main study and analyzed collectively. This will involve a blinded analysis of up to 10% of the target sample size, with an initial focus on the safety and tolerability of the IPs under evaluation.

The main clinical study

Full implementation of the clinical trial, with the primary clinical endpoint being a reduction in perceived fatigue on the Fatigue Severity Scale (FSS). This phase is an adaptive phase, with three interim analyses to evaluate therapeutic effects using the placebo arm as the baseline. The main adaptations include:

i) Discard the placebo arm if there is strong evidence of benefit.
ii) Discard active study arms that show statistically unfavorable results.
iii) Partnering with study arms of common interest from other study platforms, with the goal of obtaining responses in less time compared to conducting the study in isolation.

Randomization

Participants will be randomly assigned to one of the study arms in an equal allocation (e.g., 1:1:1). Stratified permuted-block randomization will be used for this study. Randomization will be stratified by participating primary care facility with varying block sizes of participants.

The analysis and reporting of results follow the CONSORT guidelines (www.consort-statement.org). The participant selection process and study flow will be summarized using a flow diagram. The results of the analysis of participants' demographic data and baseline outcome variables (primary and secondary) will be summarized using descriptive summary measures: expressed as mean (standard deviation) or median (minimum–maximum) for continuous variables, as appropriate, and number (percentage) for categorical variables. We will adopt an intention-to-treat principle to analyze all results.

Bayesian statistics is the standard statistical framework for this study. Statistical analyses of IPs are limited to concomitantly randomized controlled data. Sequential designs with Bayesian stopping rules are expected to be used to evaluate multiple IPs. Recruitment for an IP will be stopped before the maximum sample size using decision rules pre-specified in the statistical analysis plan (SAP). The maximum sample size and corresponding decision rules are determined a priori by dedicated statisticians and methodologists, who will remain blinded during the conduct of the study. The statistical analysis plan (SAP) was formulated to ensure that the expected one-sided Type I error is less than 0.025 for each treatment. We will also use multiple imputation to handle missing data. For all models, results will be expressed as the effect reported as the mean difference for continuous outcomes, odds ratios (OR) for binary outcomes, and hazard ratios (HR) for time-to-event outcomes, with their respective 95% confidence or credibility intervals.

All analyses will be performed using the latest version of R at the time of analysis. A detailed analysis plan will be developed prior to database lock. Any relevant safety data specific to Brazil will be immediately reported to our group for appropriate regulatory action.

The 90- and 180-day visits are post-study follow-up visits. The primary and secondary outcomes of the study are planned to be based on the D60 visit after randomization.

Analysis of Primary and Secondary Outcomes

To analyze the primary outcome of the mean fatigue score at day 60 measured by the Fatigue Severity Scale, we will use the Bayesian normal-normal model with non-informative prior distributions for the group means. This analysis will be adjusted for prespecified covariates. For all other continuous outcomes, we will use similar modeling approaches. For all binary outcomes, we will use logistic regression for analysis. The Cox proportional hazards model will be used for all time-to-event outcomes. All analyses of secondary outcomes will be exploratory in nature, without adjustment for alpha for multiple secondary analyses.

Sensitivity Analyses

	We will perform several sensitivity analyses to assess the robustness of the results, particularly for the primary outcome. These include: 1. Protocol analysis based only on participants who adhered to the protocol as described; 2. concurrent risk analysis: this analysis will adjust for death as a competitive risk for any outcome; 3. Missing data analysis: This analysis will assess the impact of missing data on the primary outcomes. 4. We will also conduct sensitivity analyses to account for any unforeseen issues that arise during the study process and that may affect the main conclusions. **Subgroup analyses** We will conduct several subgroup analyses to assess the consistency of effects across subgroups of participants: 1. Age—assumption that younger participants will benefit more than older ones. 2. Gender—assumption that women will benefit more than men. 3. Vaccination status—assumption that vaccinated individuals will benefit more than those who did not receive the vaccine. 4. Comorbidities at screening - Our hypothesis is that participants without the clinical comorbidities described above will benefit more than those without these clinical data.

Table1 : Flowchart of the procedure: Fluvoxamine, Metformin, and Placebo

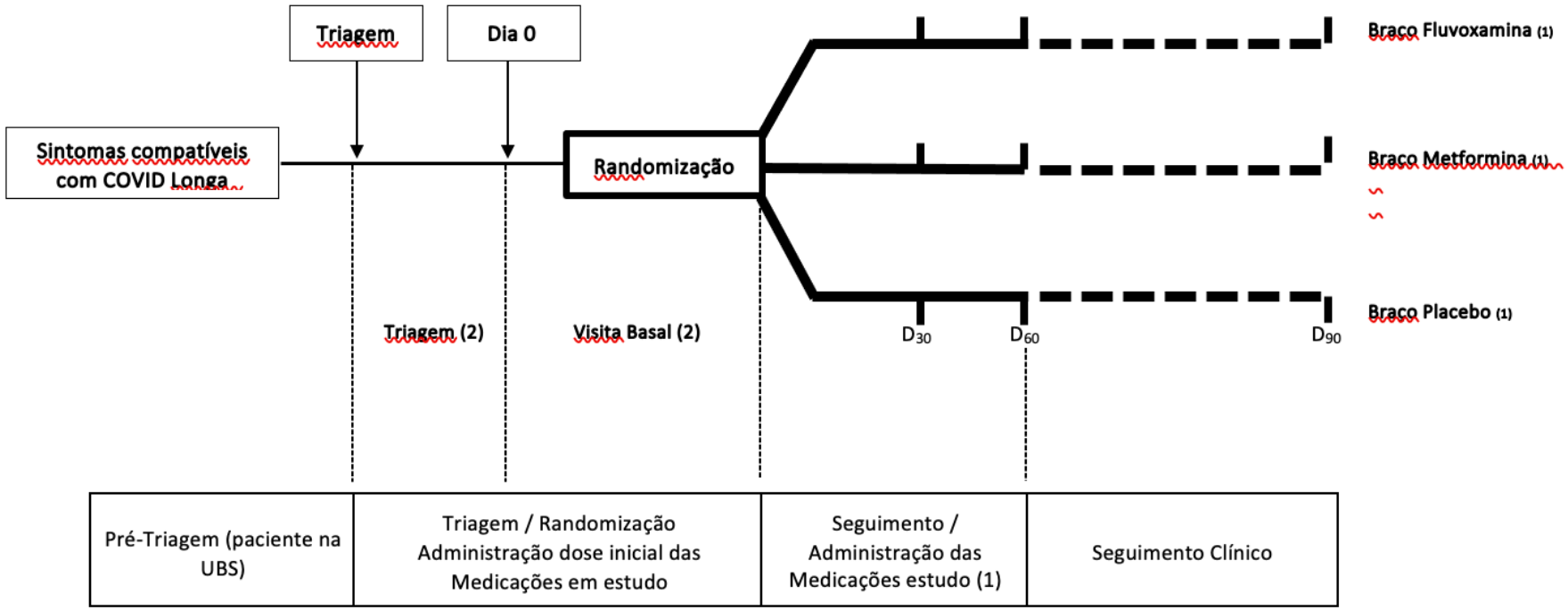
	Screening and Baseline Day 1	Day 07 ± 3 days	Day 30 ± 7 days	Day 60 ± 7 days	Days 90, 180, EoT, or early termination ± 7 days
Review of initial eligibility criteria	X(1)				
Informed consent for screening	X				
Pregnancy test	X(2)				
Second review of eligibility criteria - Conclusion and FSS scoring	X(3)				
Informed consent for participation	X				
Demographic data	X				
Medical history	X				
Comorbidities and risk factors	X				
Concomitant medications	X	X	X	X	X
Physical examination	X				
Vital signs (temperature, heart rate, respiratory rate, oxygen saturation)	X			X	
COVID-19 medical history	X				
Randomization	X				
IP administration*	X	X	X	X	
IP Adherence	X	X	X	X	

Concomitant medications	X	X	X	X	X
Fatigue Severity Scale (FSS)	X		X	X	X
Visual Analog Scale of Fatigue Intensity	X		X	X	X
EQ-5D-5L	X	X	X	X	X
Hospitalization		X	X	X	X
Mortality		X	X	X	X
Adverse events (AE)		X	X	X	X

*Fluvoxamine, metformin, and the corresponding placebo will be administered for 60 days s

Fluxograma da Pesquisa

Braços Fluvoxamina, Metformina e Placebo



1. Tratamento: Fluvoxamina, Metformina e placebo em grupos paralelos pelo período planejado. Posologia das medicações varia conforme a alocação do paciente em um braço do estudo (Para cada braço há o correspondente placebo, na mesma formulação e posologia. Medicações serão interrompidas a qualquer momento se houver evidência de reação adversa, a critério do sujeito da pesquisa ou por recomendação do DSMB (eficácia, futilidade ou segurança do participante).
2. Triagem e Randomização devem ser realizadas na mesma visita. Assegurar que o paciente seja randomizado por ocasião do atendimento.
3. As visitas subsequentes: D_{30} a D_{60}, D_{90} serão realizadas através de contato telefônico, telemedicina ou aplicativos de mídias sociais, entretanto com possibilidade de visitas presenciais, caso necessário. Em qualquer momento visitas extras de segurança poderão ser realizadas, inclusive presenciais. A visita D_{60} é considerada a visita de desfecho para a pesquisa. A visita D_{90} é considerada visita pós-estudo, para acompanhamento de eventuais complicações tardias/ persistência de sintomas relacionados ao COVID-19 e também para avaliação eventual de reações adversas tardias aos medicamentos da pesquisa. Esta será realizada através de contato telefônico. Não há previsão de visitas presenciais regulares nesta pesquisa, entretanto recomendamos que a visita D_{60} seja preferencialmente realizada presencialmente.
4. Visitas adicionais poderão ser realizadas seja por sugestão da equipe de pesquisa seja por solicitação do participante.

1 INTRODUCTION

1.1 Background

In December 2019, a series of cases of unknown etiology with symptoms similar to those of viral pneumonia began to be reported in the city of Wuhan, in Hubei Province, China[3] . These initial cases were reported among people linked to a local seafood market in Huanan ("wet market")[4] . Patients were hospitalized with this viral pneumonia, and bronchoalveolar lavage fluid samples were collected from three patients, from which a new coronavirus, named 2019-nCoV, was isolated. Evidence for the presence of this virus included its identification in the bronchoalveolar lavage fluid of three patients via genome sequencing, direct PCR, and culture. The disease likely caused by this CoV was named "novel coronavirus-infected pneumonia." The complete genomes were submitted to GISAID. Phylogenetic analysis revealed that 2019-nCoV belonged to the genus betacoronavirus, which includes coronaviruses (SARS-CoV, SARS-like bat CoV, and others) discovered in humans, bats, and other wild animals[3] .

As the number of cases increased, on January 30, 2020, the outbreak was declared a Public Health Emergency of International Concern. As of January 31, 2020, there were 9,826 confirmed cases of 2019-nCoV worldwide[5] . On that same day, the first two cases of 2019-nCoV were reported in Italy, and both had a history of travel to the city of Wuhan, China. There were also confirmed cases in 18 other countries besides Italy, for a total of 19 countries outside of China[5] .

As of February 11, 2020, 43,103 cases had been confirmed (42,708 of them in China) and 1,018 deaths had been reported. On that same day, the World Health Organization (WHO), in collaboration with its departments (the World Organization for Animal Health and the Food and Agriculture Organization of the United Nations), named the disease COVID-19 (short for "coronavirus disease 2019")[6] . On the same day, the Coronavirus Study Group (CSG) of the International Committee on Viral Taxonomy proposed naming the new coronavirus SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2)[7] .

On March 11, 2020, the World Health Organization declared COVID-19 a global pandemic[8,9] .

1.2 Mechanisms of Infectivity

This global health emergency has intensified research efforts to better understand the pathogenesis, clinical manifestations, and outcomes of people affected by this new viral strain. It is known that the “spike proteins” of coronaviruses, including SARS-CoV-2, interact with Angiotensin-Converting Enzyme 2 (ACE2) and type II transmembrane serine proteases to invade cells[10,11] . Thus, cells expressing ACE2, including pneumocytes and ciliated lung cells of the tracheobronchial tree, cardiac endothelial cells, intestinal mucosal cells, and renal epithelial cells, may be affected, which could partly explain the multi-organ dysfunction observed in participants[12] . Under physiological conditions, ACE2 acts as a natural antagonist of the renin-angiotensin-aldosterone system (RAAS) by degrading angiotensin II and thereby producing angiotensins 1–7, which limit the vasoconstrictive capacity of angiotensin I. Angiotensins 1 through 7 have protective pulmonary effects by attenuating the inflammatory response[13] . In fact, as observed in recent SARS-CoV epidemics (SARS and MERS) and recently identified in genetic studies of SARS-CoV-2, the inhibition of ACE2 transmembrane receptor expression resulting from viral infection occurs through their blockade by “spike proteins.” This abrupt reduction in ACE2 activity in lung cells is a critical factor in the resulting pulmonary complications, given its important inhibitory effect on pulmonary inflammatory mediators, thereby reducing pulmonary edema and the unwanted amplification of the inflammatory response resulting from COVID-19[7] .

1.3 Immune Response in COVID-19

In the early stage of SARS-CoV-2 infection, the immediate immune response triggered in response to the virus can effectively eliminate it[14] . Stimulation of innate immune cells leads to the production of inflammatory mediators, which, together with the complement system, exert antiviral effects in the early stage[15] . Viruses have evolved to employ various strategies to evade this innate immune response. For example, viruses can evade the complement system by removing antibody-antigen complexes from cell surfaces, downregulating Fc receptor expression, or mimicking the regulatory component of the complement[16,17] . Studies have shown that the replication efficiency of SARS-CoV-2 in releasing infectious viral particles and “deceiving” the innate immune response induced in human intestinal tissues is more intense than that of SARS-CoV infection[18] .

Elevated cytokine levels commonly occur in critically ill COVID-19 patients, including IL-6, IL-2R, IL-10, and tumor necrosis factor-alpha [19] . Autopsy data revealed intense generalized inflammatory activation involving the gastrointestinal tract, with intense neutrophilic and reticular cell activity. Despite this inflammatory activation, cells infected with the SARS-CoV-2 virus were rarely identified, suggesting a maladaptive immune response (cytokine storm[20]). In these situations, the

direct cytopathological effects of SARS-CoV-2 induce apoptosis (a highly inflammatory form of programmed cell death) and release cellular mediators that culminate in the amplification of the inflammatory cascade characterized by the secretion of pro-inflammatory cytokines and chemokines that attract monocytes, macrophages, and T cells, leading to extensive tissue damage[21] . In these situations, macrophages frequently communicate with SARS-CoV-2 targets via chemokines and phagocytic signals[22] . This inflammatory process can regulate $_{ACE2}$ expression in macrophages, leading to an intense amplification of tissue damage[23] .

One of the potential catastrophic effects of SARS-CoV-2 infections is cytokine storms. In fact, concentrations of circulating pro-inflammatory factors, such as IL-1, IL-6, and TNF-α, are strongly associated with ICU admission and mortality in patients with COVID-19. Studies of the first pandemic coronavirus, SARS-CoV, have shown that viral proteins play an active role in the processing and release of two specific pro-inflammatory cytokines, IL-1β and IL-18, by increasing NF-κB transcriptional activity and promoting the formation of the NLRP3 inflammasome[24] (Figure 10).

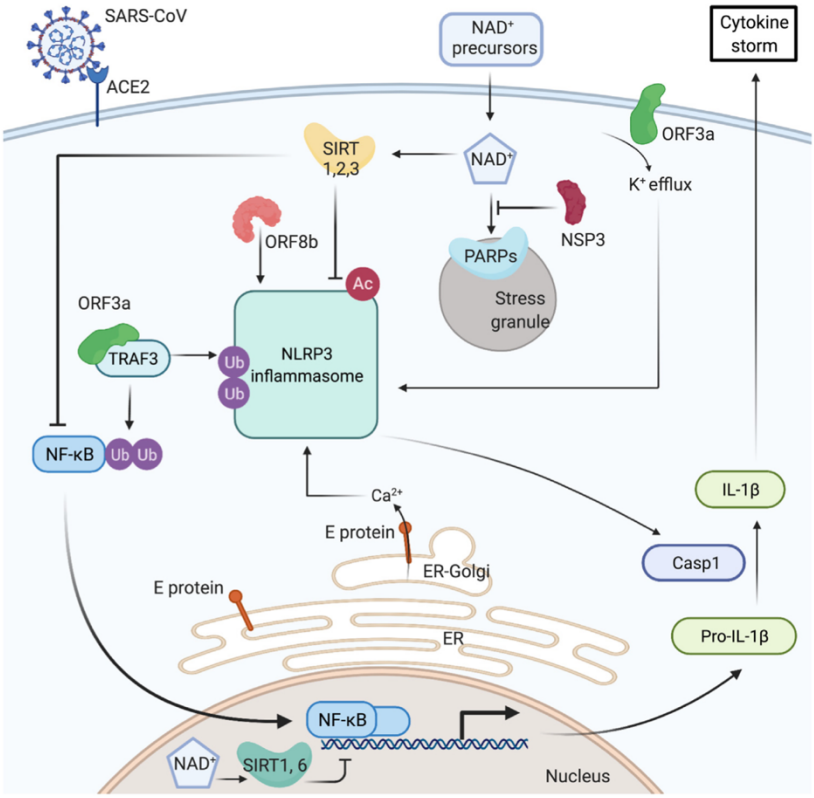


Figure 4 - Regulation of the NLRP3 inflammasome by SARS-CoV infection and NAD+. Several proteins encoded by SARS-CoV promote NLRP3 inflammasome activity and the release of pro-inflammatory cytokines. ORF3a activates NF-κB through TRAF3-dependent ubiquitination, which facilitates ASC cluster formation and

NLRP3 inflammasome assembly [86]. ORF3a also possesses transmembrane domains and ion channel activity that drives a K+ efflux [85]. Protein E is located in the ER-Golgi compartment and promotes Ca2+ efflux [87,88]. ORF8b interacts directly with NLRP3. All of these mechanisms activate the inflammasome [89]. Host cells SIRT1, SIRT2, and SIRT3 suppress the NLRP3 inflammasome [94-98]. SIRT1 deacetylates NF-κB, suppressing its activity, and reduces oxidative stress, thereby decreasing inflammasome activation. SIRT2 deacetylates NLRP3. SIRT3 suppresses mitochondrial ROS production, thereby decreasing inflammasome activation. SIRT6 reduces inflammation through deacetylation of H3K9 in the promoters of NF-κB target genes [99]. PARPs promote the formation of antiviral SGs through ADP-ribosylation of SG components. Abbreviations: Ac, acetylation; ACE-2, angiotensin-converting enzyme 2; ASC, apoptosis-associated spot-like protein containing a caspase recruitment domain; Casp1, caspase-1; ER, endoplasmic reticulum; NF-κB, nuclear factor κB; NLRP3, NOD-, LRR-, and pyrin-domain-containing protein 3; ORF, open reading frame; SARS-CoV, severe acute respiratory syndrome coronavirus 1; SIRT, sirtuin; TRAF3, TNF receptor-associated factor 3; Ub, ubiquitination.

SARS-CoV proteins that facilitate these processes include open reading frame (ORF) 3a, envelope (E) protein, and ORF8b[25] . ORF3a mediates NF-κB activation through TRAF3-dependent ubiquitination of an NF-κB inhibitory subunit and mediates the formation of inflammasome ASC subunit aggregates, which accompanies NLRP3 inflammasome assembly in human cells. Furthermore, ORF3a possesses transmembrane domains and ion channel (IC) activity that drives K+ efflux, which further promotes NLRP3 inflammasome activation. In fact, IL-1β secretion was completely blocked when BMDMs, stimulated with lentiviruses expressing SARS-CoV ORF3a, were treated with K+-rich medium. The SARS-CoV E protein also promotes inflammasome activation through its intracellular activity; it forms lipid-protein channels in the endoplasmic reticulum (ER)-Golgi intermediate compartment, and the resulting Ca2+ stimulates NLRP3 inflammasome activation.

Like SARS-CoV, SARS-CoV-2 can also activate the inflammasome. Active NLRP3 inflammasomes were found in peripheral blood mononuclear cells (PBMCs) and tissues from deceased COVID-19 patients following autopsy, along with higher concentrations of serum IL-18 that correlated with the severity of COVID-19. SARS-CoV-2 can activate the inflammasome through mechanisms similar to those of SARS-CoV-[26] .

NAD+ may contribute to the resolution of inflammation and to limiting or preventing the effects of cytokine storms. In the presence of reduced NAD+ concentrations, there is an enhancement of cytokine and pro-inflammatory activation induced by SARS-CoV and SARS-CoV-2. Similarly, low

NAD+ concentrations may exacerbate the severity of COVID-19; therefore, increasing levels of this metabolite in at-risk populations is a potential therapeutic strategy to prevent severe forms of COVID-19[27] .

1.4 Suppression of NLRP3 inflammasome activation

Inflammasomes are protein complexes that are activated in response to certain pro-inflammatory signals and induce the auto-activation of caspase-1; this protease, in turn, cleaves pro-interleukin-1β and pro-interleukin-18 to generate the active forms of these mediators, which exert a crucial pro-inflammatory and pro-apoptotic effect in many pathologies[28,29] . Inflammasomes can also induce a type of cell death known as pyroptosis; this results from caspase-1-mediated cleavage of the Gasdermin-D protein, which subsequently forms a plasma transmembrane channel that allows the extracellular efflux of cytokines, such as interleukin-1β, and which can also induce edema and cell death[30] . A variety of different types of inflammasomes have been characterized; these include the NLR subset (NLRP1, NLRP3, NAIP/NLRC4), as well as the AIM2 and IFI16 inflammasomes. Given that the NLRP3 inflammasome is closely associated with the uncontrolled activation of the inflammatory cascade in COVID-19, we will focus on this inflammasome.

The NLRP3 inflammasome consists of the NLRP3 protein interacting with ASC and caspase-1 proteins, along with several accessory proteins, including NEK7[31] . The formation of NLRP3 inflammasomes typically requires a “priming” step, in which the activated transcription factor Nuclear Factor kappa beta (NF-kappaB) drives the increased expression of NLRP3 and pro-interleukin-1β and -18. Subsequent activation of NLRP3 inflammasomes, in which the NLRP3/ASC/caspase-1 complex is formed, is typically triggered by oxidative stress, a drop in intracellular potassium, and/or lysosomal rupture, which releases cathepsins[32,33] . The role of oxidative stress in triggering NLRP3 inflammasome assembly is well characterized. This sequence requires an interaction between the thioredoxin-interacting protein (TXNIP) and NLRP3[34] ,[35] .

1.5 Suppression of the NLRP3 inflammasome via phase 2 induction

Phase 2 inducers are agents that trigger increased expression of a wide range of antioxidant, detoxifying, and cytoprotective enzymes. Many of them do this by covalently interacting with the cysteine groups of Keap1, a protein that binds to the transcription factor Nrf2, retaining it in the cytoplasm and promoting its proteasomal degradation[36,37] . When phase 2 inducers—or their

electrophilic metabolites—bind to Keap1, Nrf2 is released to migrate to the nucleus and promote the transcription of many cytoprotective enzymes, whose gene promoters contain antioxidant response elements capable of binding to Nrf2. Of fundamental importance to our discussion is the fact that phase 2 induction increases the expression of both thioredoxin and thioredoxin reductase, as well as glutathione peroxidase, which is capable of eliminating hydrogen peroxide[38,39,40] . Phase 2 nutraceutical inducers that have demonstrated significant clinical utility include lipoic acid, ferulic acid, melatonin, sulforaphane, and phycocyanobilin[41,42,43,44] .

In the early stages of SARS-CoV-2 infection, an appropriate immune response is initiated against the virus, as occurs against similar coronavirus infections such as SARS-CoV-1 and MERS-CoV[45,46] . In a subset of participants, the course of the disease may progress to an immunodysregulated state characterized by systemic hyperinflammation ("cytokine storm syndrome")[47,48,49,50] . This state may manifest clinically as ARDS, shock, and multiple organ failure. The resulting mortality rate is equal to or greater than 50% in this population[51,52] , making it essential to seek interventions that can effectively address this subgroup of patients. Current approaches are limited to immunosuppressive therapies, many of which are still experimental, in participants who have already developed advanced disease[53,54] . Disease-modifying therapies that address the underlying pathophysiology and prevent progression to the hyperinflammatory state will be essential to mitigate COVID-19-related morbidity and mortality at the population level[52] .

Biomarkers of advanced stages and poor outcomes in COVID-19 support immunopathology models and suggest potential intervention strategies. Absolute counts and relative proportions of immune cells and lymphocyte subsets are aberrant in COVID-19, especially in severe cases[Erro! Indicador não definido.,55(,)56,57,58,59,60,61] . Inflammatory cytokines, chemokines, and other markers of inflammation including IL-2, IL-6, IL-7, IL-8, soluble IL-2 receptor, interferon-γ-inducible protein 10, monocyte chemotactic protein 1, colony-stimulating factor for granulocytes, macrophage inflammatory protein 1-α, tumor necrosis factor-α, C-reactive protein, ferritin, and D-dimer, among others, are also elevated in severe cases[Erro! Indicador não definido.,Erro! Indicador não definido.,Erro! Indicador não definido.,Erro! Indicador não definido.(,)Erro! Indicador não definido.,Erro! Indicador não definido.,Erro! Indicador não definido.,Erro! Indicador não definido.,62,63,64,65] . IL-6 specifically differs between non-survivors and survivors and is predictive of COVID-19 severity and in-hospital mortality[Erro! Indicador não definido.,Erro! Indicador não definido.,Erro! Indicador não definido.] . The levels of these markers mirror those observed in the cytokine storm induced by SARS-CoV-1 and MERS-CoV infection[66,67,68,69] .

The cytokine storm is associated with ARDS, the leading cause of mortality in SARS and MERS[70,71] . COVID-19 cytokine profiles also resemble the state of hyperinflammation seen in primary hemophagocytic lymphohistiocytosis (HLH), a hyperinflammatory syndrome caused by underlying defects in perforin signaling pathways, and macrophage activation syndrome (MAS) observed in a subset of participants with autoimmune rheumatic disease[Erro! Indicador não definido.,72,73,74,75] . Furthermore, the profile of immune dysregulation in COVID-19 shares similarities with CRS seen as an adverse effect of cellular immunotherapies, including CAR-T cell therapy[76,77,78,79] .

Data from randomized controlled trials and retrospective case series of participants with severe or critical COVID-19 treated with tocilizumab or siltuximab suggest that inhibition of the IL-6 signaling axis may be effective[Erro! Indicador não definido., 80] . However, preliminary findings from a randomized, double-blind, placebo-controlled trial of sarilumab in COVID-19 suggest that targeting the IL-6 signaling pathway in participants with advanced disease may not be as effective as suggested by observational data[81] . Although immunosuppressive treatments likely play an important role in COVID-19[Erro! Indicador não definido.] , the considerable cost, limited availability, and potential for serious adverse events limit the application of biologic therapies targeting different cytokine axes in COVID-19.

1.6 Thrombosis, coagulopathy, sepsis, and gastrointestinal lesions

Some recent cases have reported acute diarrhea, bleeding, and acute mesenteric thrombosis in participants infected with the novel coronavirus[82,83] . According to a recent study, intestinal abnormalities were a common finding (31%) on abdominal imaging in participants with COVID-19, and participants who required laparotomy frequently presented with histological ischemia due to thrombosis of small blood vessels[84] . The direct attack of SARS-CoV-2 on the vascular endothelial system eventually leads to a vicious cycle between sepsis and increased cytokine levels. Vascular damage associated with endothelial cells and the increase in circulating cytokines together lead to a state of blood hypercoagulability, thereby inducing the occurrence of intestinal coagulation disorders.

Elevated levels of D-dimer and fibrinogen in many participants with COVID-19 may not only be a common cause of peripheral and pulmonary thrombosis, but also the primary cause of intestinal hypercoagulability and ischemic events[85,86] . Among hospitalized participants with COVID-19, the proportion of participants with elevated D-dimer levels was as high as 47%[87] . Compared to participants with coagulopathy, the typical manifestation in participants with COVID-19 was an

increase in D-dimer concentration and the induction of mononuclear cells through elevated IL-6, thereby expressing tissue factor, which in turn induces coagulation activation and thrombin generation. TNF-alpha and IL-1 are the main mediators that inhibit the endogenous anticoagulation pathway.

1.7 The Long COVID Phenomenon

Shortly after the onset of the global COVID-19 pandemic, reports emerged showing that some individuals infected with SARS-CoV-2 developed persistent symptoms and new health problems that arose long after the acute phase of infection and could not be explained by other factors[88] . The community of researchers who first recognized and reported this new syndrome used the term “Long COVID-19” to describe the post-acute and chronic sequelae of SARS-CoV-2 infection[89] . Long COVID (sometimes called “post-acute sequelae of COVID-19”) is a multisystemic condition comprising often severe symptoms that follow infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Long COVID can affect people of all ages—children, young adults, and older adults—and across all genders, races, and ethnicities, regardless of baseline health status[90] . It is important to note that this syndrome does not only affect participants who had severe COVID-19, but is also observed in individuals who were asymptomatic or mildly symptomatic during the acute phase of SARS-CoV-2 infection.

1.8 Definition of Long COVID

Although there is still no formal clinical definition of this nosological entity, the findings attributed to this condition include persistent shortness of breath, sleep disturbances, hyperlipidemia, fatigue, gastroesophageal reflux disease (GERD), cough, muscle weakness, joint pain, thromboembolism, renal disorders, neurological deficits, and cardiopulmonary abnormalities[91,92,93] .

LONG COVID is distinguished from acute COVID-19 primarily by the timing of symptoms relative to the onset of the disease, with acute COVID-19 defined as symptoms persisting for up to four weeks after onset, and LONG COVID restricted to symptoms that persist or develop more than two months after the onset of symptoms. It is estimated that 10–15% of convalescent COVID-19 patients may develop Long COVID[94] , with 40% of these individuals reporting that their symptoms significantly affect their ability to perform basic daily tasks. Given the high prevalence of this post-COVID-19 clinical condition, Long COVID has become a major public health concern.

Many clinical definitions have been proposed for the long-term, often permanent, sequelae of COVID-19[95,96] . Using the Delphi methodology, involving scientists and participants from all regions of the world, the World Health Organization (WHO) defined Long COVID as a condition occurring in individuals with a history of probable or confirmed SARS-CoV-2 infection , which typically develops three months after the onset of symptoms, persists for at least two months, and cannot be explained by alternative diagnoses[97] . Symptoms may develop after initial recovery from acute SARS-CoV-2 infection or persist from the original illness, fluctuate, or even relapse over time, and generally affect daily functioning.

A core outcome for clinical research has also been defined[98] , encompassing 4 domains (physiological or clinical outcomes, life-impact outcomes, survival, and outcomes from previous endpoints), broken down into 11 endpoints: cardiovascular functioning, symptoms, and conditions; fatigue or exhaustion; pain; nervous system functioning, symptoms, and conditions; cognitive functioning, symptoms, and conditions; mental functioning, symptoms, and conditions; respiratory functioning, symptoms, and conditions; post-exercise symptoms and conditions; changes in work, occupation, and studies; survival; and recovery from previous outcomes.

1.9 Immune dysregulation, inflammatory storm, and persistent inflammation associated with Long COVID

There is growing evidence that LONG COVID is accompanied by dysregulated and persistent inflammation in multiple organs[99,100,101] . Elevated serum levels of inflammatory biomarkers such as C-reactive protein, TNFα, IFNγ, and IL-6 were observed in convalescent COVID-19 participants who progressed to LONG COVID, with the intensity of these markers directly associated with the number of post-acute COVID-19 syndrome (PACS) symptoms reported by the patient. Phetsouphanh et al. described persistent immune dysfunction in individuals 8 months after mild COVID-19, characterized by detectable signatures of a highly activated innate immune system and elevated expression of both type I and type III interferons, as well as elevated concentrations of CXCL9, CXCL10, IL-8, and sTIM-3[102] . It is postulated that this persistent inflammatory “scar” may drive tissue damage, endothelial dysfunction, hypoxia-induced tissue injury, and activation of pathogenic effector lymphocyte subsets[103 , 104] . A consistent finding associated with chronic inflammatory dysregulation has been the near-universal detection of elevated plasma levels of IFN-γ and IL-2, suggesting that uncontrolled immune activation may contribute to the persistence of LONG COVID symptoms following the acute episode of viral infection. Similarly, this inflammatory activation is an indicator of endothelial dysfunction, which may be followed by vascular injury as a

consequence of this production of circulating cytokines, generally released by activated CD8+ cytotoxic lymphocytes[105] .

In addition to triggering uncontrolled inflammation, it has also been suggested that both severe COVID-19 and LONG COVID are accompanied by the development of an auto-reactive immune response. The resulting production of autoantibodies can be documented through the development of humoral immunity against SARS-CoV-2[106,107,108] .

1.10 Clinical manifestations, symptoms, and pathogenesis

Long COVID generally manifests as fatigue and neurocognitive impairment (also known as “brain fog”); however, hundreds of biomedical findings have been documented, with many participants presenting dozens of symptoms across multiple organ systems (Figure1)92 . Long COVID encompasses various adverse outcomes, with common conditions of recent onset including cardiovascular, thrombotic, and cerebrovascular diseases[90] , type 2 diabetes[89] , myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)[109] , and dysautonomia, particularly postural orthostatic tachycardia syndrome (POTS)[110] .

Symptoms can last for years[111] and, particularly in cases of ME/CFS and recent-onset dysautonomia, are expected to be lifelong[112] . With significant proportions of individuals with long COVID unable to return to work[92] , the scale of new individuals with disabilities is contributing to labor shortages[113] .

There are likely to be several, potentially overlapping, causes of long COVID. Several hypotheses for its pathogenesis have been suggested, including persistent reservoirs of SARS-CoV-2 in tissues[114] ; immune dysregulation[115] with or without reactivation of underlying pathogens, including herpesviruses such as Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6), among others[116] ;impacts of SARS-CoV-2 on the microbiota, including the virome[117] ; autoimmunity[118] and immune system priming through molecular mimicry[119] ; microvascular blood coagulation with endothelial dysfunction[120] ; and dysfunctional signaling in the brainstem and/or vagus nerve[121] .

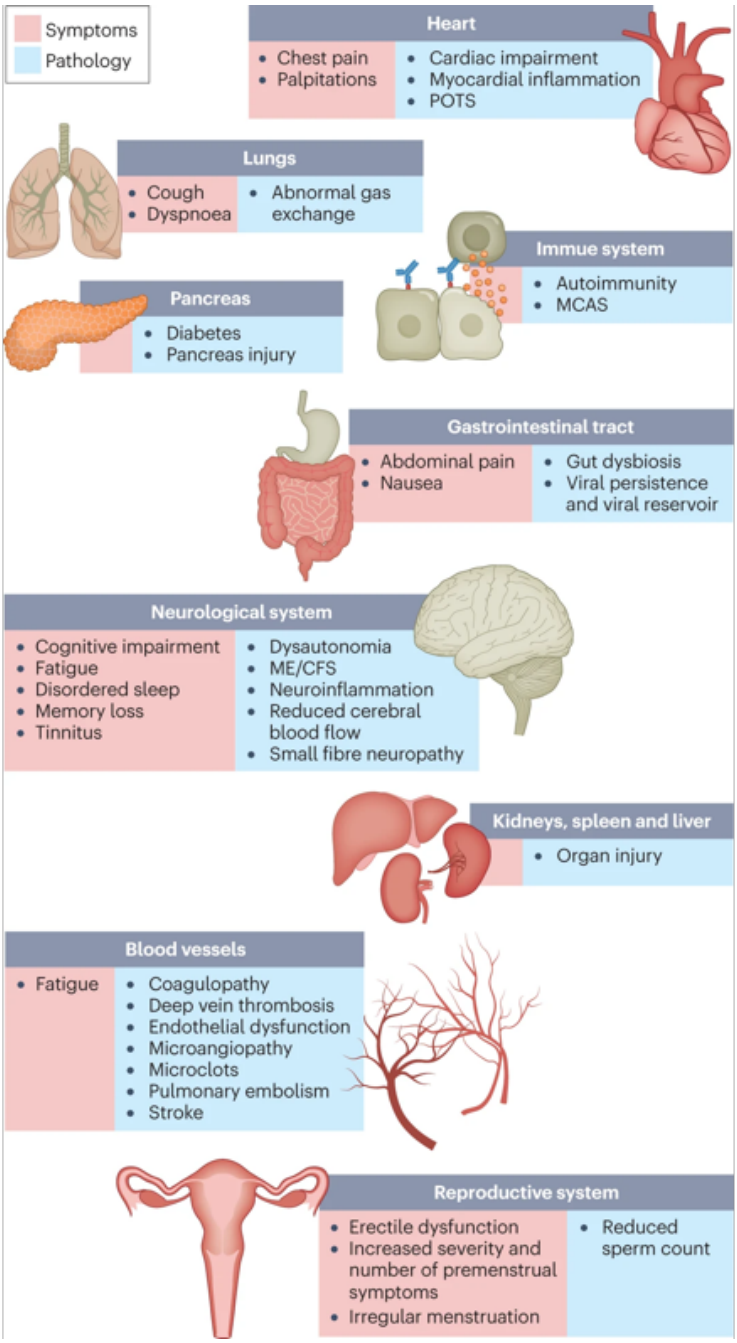


Figure1 : Long COVID symptoms and their impacts on various organs with different pathologies

1.11 Risk Factors

Potential risk factors include female gender, type 2 diabetes, EBV reactivation, the presence of specific autoantibodies[118] , connective tissue disorders[122] , attention-deficit/hyperactivity disorder, chronic urticaria, and allergic rhinitis[123] , although up to 33% of individuals with long COVID have no

identified pre-existing conditions. A higher prevalence of long COVID has been reported in certain ethnic groups, including people of Hispanic or Latino origin[124] . Socioeconomic risk factors include lower income and an inability to rest adequately in the first few weeks after developing COVID-19[125] . Prior to the emergence of SARS-CoV-2, various viral and bacterial infections were known to cause post-infectious illnesses, such as ME/CFS[126] , and there is evidence that long COVID may share their mechanistic and phenotypic characteristics[127] . Furthermore, dysautonomia has been observed in other post-viral illnesses and is frequently observed in long COVID[92] .

1.12 Diagnostic Tools

Although diagnostic tools exist for some components of long COVID (e.g., tilt table tests for POTS[128] and MRI scans to detect cardiovascular involvement)[129] , diagnostic tools for long COVID are, for the most part, still under development, including imaging tests to detect microclots[130] , corneal microscopy to identify small fiber neuropathy[88] , new QRS complex fragmentation on electrocardiograms as an indicator of cardiac injury, and the use of hyperpolarized MRI to detect abnormalities in pulmonary gas exchange[131] . Based on the tests offered as standard care, results for participants with long COVID are generally normal; many providers are unaware of symptom-specific tests and diagnostic recommendations from the ME/CFS community. Early biomarker research suggests that levels of extracellular vesicles[132] and/or immune markers indicating high cytotoxicity[133] could be indicative of long COVID. Interestingly, dogs can identify individuals with long COVID based on sweat samples[134] .

Biomarker research in MS/CFS may also be applicable to long COVID, including electrical impedance blood tests, saliva tests, erythrocyte deformation, sex-specific plasma lipid profiles, and variables related to isocapnic buffering[135] . The importance of developing and validating biomarkers that can be used for the diagnosis of long COVID cannot be overstated—they will not only be useful for establishing the diagnosis but also for objectively defining treatment responses.

Specific functional assessment tools can be applied according to the patient's condition. The Patient-Reported Outcome Measurement Information System,[38] the Post-COVID-19 Function Status Scale[136] , and EuroQol-5D can be used to assess the functional status or quality of life of the patient. The modified Medical Research Council Dyspnea Scale[137] can be used in participants with dyspnea. For neurological conditions, the Montreal Cognitive Assessment[138] , Compasso 31[139] , and the Neurobehavioral System Inventory[140] are useful tools. For psychiatric conditions, the Generalized Anxiety Disorder 7, the Patient Health Questionnaire 9, the PTSD Symptom Scale, the Post-Traumatic Stress Symptom Screening, the DSM-5 PTSD Checklist, the Revised Impact of Event Scale, the Hospital Anxiety Scale, and the Depression Scale[141] can be used.

1.13 Therapeutic Options

Although there are currently no widely effective treatments for long COVID, treatments for specific symptoms have been effective for certain subgroups of the population. Many strategies used for MS/CFS are effective for individuals with long COVID, including stimulation[124] and symptom-specific pharmacological options (e.g., β-blockers for POTS, low-dose naltrexone for neuroinflammation[142] and intravenous immunoglobulin for immune dysfunction) and non-pharmacological options (including increased salt intake for POTS, cognitive stimulation for cognitive dysfunction, and elimination diets for gastrointestinal symptoms)[143] . Low-dose naltrexone has been used for many conditions, including MS/CFS, and has also shown promise in the treatment of long COVID[143,144] . $H_1$and H_2antihistamines, often following protocols for mast cell activation syndrome and particularly involving famotidine, are used to relieve a wide range of symptoms. Another drug, BC007, has the potential to treat autoimmunity by neutralizing levels of G protein-coupled receptor autoantibodies[145] . Anticoagulant regimens are a promising way to treat abnormal coagulation. In one study, symptom resolution was observed in all 24 participants who received triple anticoagulant therapy[130, 146] . Apheresis has also shown promise in alleviating prolonged COVID-19 symptoms; it has been theorized that it helps remove microclots and has been shown to reduce autoantibodies in ME/CFS[147] . However, it is quite expensive and its benefits are uncertain. Some supplements have shown promise in the treatment of long COVID and MS/CFS, including coenzyme Q_{10} and D-ribose, and may warrant further study[148] .

Pilot studies and case reports have revealed additional treatment options worth exploring. One case report noted the resolution of long COVID following treatment with the antiviral Paxlovid[149] , and a study investigating the treatment of acute COVID-19 with Paxlovid showed a 25% reduction in the incidence of long COVID[150] . A small study of sulodexide in individuals with endothelial dysfunction observed a reduction in symptom severity[151] . Pilot studies of probiotics have indicated potential for alleviating gastrointestinal and non-gastrointestinal symptoms[152] . An initial study found that Pycnogenol statistically significantly improved physiological measures (e.g., reduced oxidative stress) and quality of life (indicated by higher scores on the Karnofsky Performance Scale Index)[153] , consistent with the hypothesis based on success in other clinical studies.

Taken together, current treatment options are based on small-scale pilot studies on long-haul COVID-19 or on what has been effective for other diseases; several other studies are ongoing[154] .

There is a wide range of potential treatment options for ME/CFS that encompass various mechanisms, including enhancing natural killer cell function, removing autoantibodies, immunosuppressants, antivirals for reactivated herpesviruses, antioxidants, mitochondrial support, and mitochondrial energy generation[155] ; most need to be clinically tested, which should occur urgently. Many newer treatment options remain under-explored, including anticoagulants and SARS-CoV-2-specific antivirals, and the lack of funding is a significant limitation to conducting robust trials.

1.14 Fluvoxamine

Fluvoxamine is a selective serotonin reuptake inhibitor (SSRI) and an S_1R receptor agonist[156] . The rationale for considering the use of fluvoxamine in participants with COVID-19 is that S_1R receptor agonists may attenuate excessive inflammation in participants with COVID-19. This and other potential mechanisms by which fluvoxamine may act in COVID-19 are summarized below.

1.14.1 Anti-inflammatory effects via the S_1R-IRE pathway

S_1R is an endoplasmic reticulum (ER) chaperone protein involved in many cellular functions, including the regulation of the ER stress response / unfolded protein response (UPR) and inflammation[157] . The S_1R protein has been shown to inhibit the inositol-requiring ER stress sensor enzyme 1α ($_{IRE1}$) $_{via\ XBP1\text{-}mediated}$ splicing, a key regulator in cytokine production[158] . These anti-inflammatory effects may be the most likely explanation for the beneficial effects of fluvoxamine. In COVID-19, an excessive inflammatory process known as a “cytokine storm” can contribute to the worsening of symptoms and cardiopulmonary complications, which can sometimes occur around the second week of the disease. Fluvoxamine may attenuate this excessive inflammatory response.

In a 2019 study conducted by Rosen, fluvoxamine demonstrated benefits in preclinical models of inflammation and sepsis[159] . In one model, mice were exposed to Toll-like receptor 4 ($_{TLR4}$), lipopolysaccharide (LPS), which can trigger an inflammatory response. In another model, a fecal concentrate was injected, triggering an infection and an inflammatory response that is generally sublethal. Mice lacking S_1R receptors showed excessive increases in cytokine levels and significantly reduced survival under either of these conditions, suggesting that these receptors inhibit the exacerbated inflammatory response. Non-genetically manipulated mice exposed to the same inflammatory triggers exhibited reduced cytokine levels and increased survival when treated with fluvoxamine (an S_1R agonist). In investigating the mechanism underlying this effect, the authors demonstrated that S_1 R receptors inhibit IRE_1 activity, which in turn prevents excessive cytokine

production. In an experiment using human peripheral blood, they also showed that fluvoxamine can reduce LPS-induced cytokine production by human cells. In the case of COVID-19, the S1R agonist action of fluvoxamine may have a similar ability to reduce the excessive inflammatory response induced by viral infection, thereby reducing inflammation-mediated organ damage.

1.14.2 Antiviral action through effects on lysosomes, autophagy, and/or endocytosis.

Coronaviruses utilize cathepsin-like proteases, present in the late endosome, to facilitate entry into the cell and remodel phagosomes and endoplasmic reticulum membranes, transforming them into sites of “viral replication”[160,161] . Both processes require the stimulation of endocytosis and autophagy-phagosome-mediated pathways, followed by the termination of autophagy prior to lysosomal fusion. It has been demonstrated that the SARS-CoV-2 proteins Nsp6, Nsp2, Orf7b, and Orf9b localize to and modulate components of the autophagy pathway[162 , 163] . It has been demonstrated that Nsp6 additionally physically associates with S_1R[164] . Critically, S_1R not only drives early-stage autophagy via the IRE_1/UPR pathway, but is also essential for lysosomal fusion and for completing autophagy, likely by interacting with components of the SNARE complex[165] . It is possible that activation of S_1R by fluvoxamine may overcome Nsp6 inhibition of S1R to allow autophagy to eliminate SARS-CoV-2. Others have also recognized targeting the autophagy pathway as a promising strategy for treating SARS-CoV-2[166,167] .

Chemically, fluvoxamine is a cationic amphiphilic drug (CAD) with a log P of 3.1 and a pKa of 9.4, and, along with a variety of antipsychotic and antihistamine drugs, it accumulates preferentially in lysosomes. Perhaps because of this, fluvoxamine reaches higher concentrations in the lungs (which are rich in lysosomes) than in the brain[168] . In the case of COVID-19, this may enhance the effects of treatment on the airway epithelium[169] . At high doses (10 μM), CADs including fluvoxamine have been shown to inhibit lysosomal acid sphingomyelinase and cause drug-induced phospholipidosis. This nonspecific activity can globally disrupt lipid homeostasis, which in turn modulates autophagy via the mTOR nutrient sensing pathway[170,171] .

1.14.3 Antiviral effects and prevention of organ damage through regulation of the ER stress/UPR response.

Some viruses hijack the ER stress/UPR response to achieve viral functions, and a series of studies have suggested that drugs targeting the ER stress/UPR response may be beneficial in the treatment of COVID-19[172,173,174] . S_1R agonists (such as fluvoxamine) regulate ER-associated stress.

The effects of S_1R ligands during ER-mediated stress and other ER functions may reduce organ dysfunction/damage[175,176] .

1.14.4 Antiplatelet effects (common to all SSRIs)

Platelet hyperactivity may contribute to pathophysiological processes leading to thrombotic complications in COVID-19. SSRIs may inhibit platelet activation, which may reduce the risk of thrombosis, and these antiplatelet effects may be cardioprotective[177,178] .

1.14.5 Elevated melatonin levels

The SARS-CoV-2 virus can activate the NLRP3 inflammasome, which may contribute to the cytokine storm[179,180] . Melatonin may act on this NLRP3 pathway to reduce inflammation[181,182] . Fluvoxamine inhibits melatonin metabolism, and therefore may increase melatonin levels in the body, which may be beneficial in COVID-19[183] .

A summary of the potential benefits of fluvoxamine use in COVID-19 was described by one of the researchers associated with this arm and is summarized in the figure below[184] .

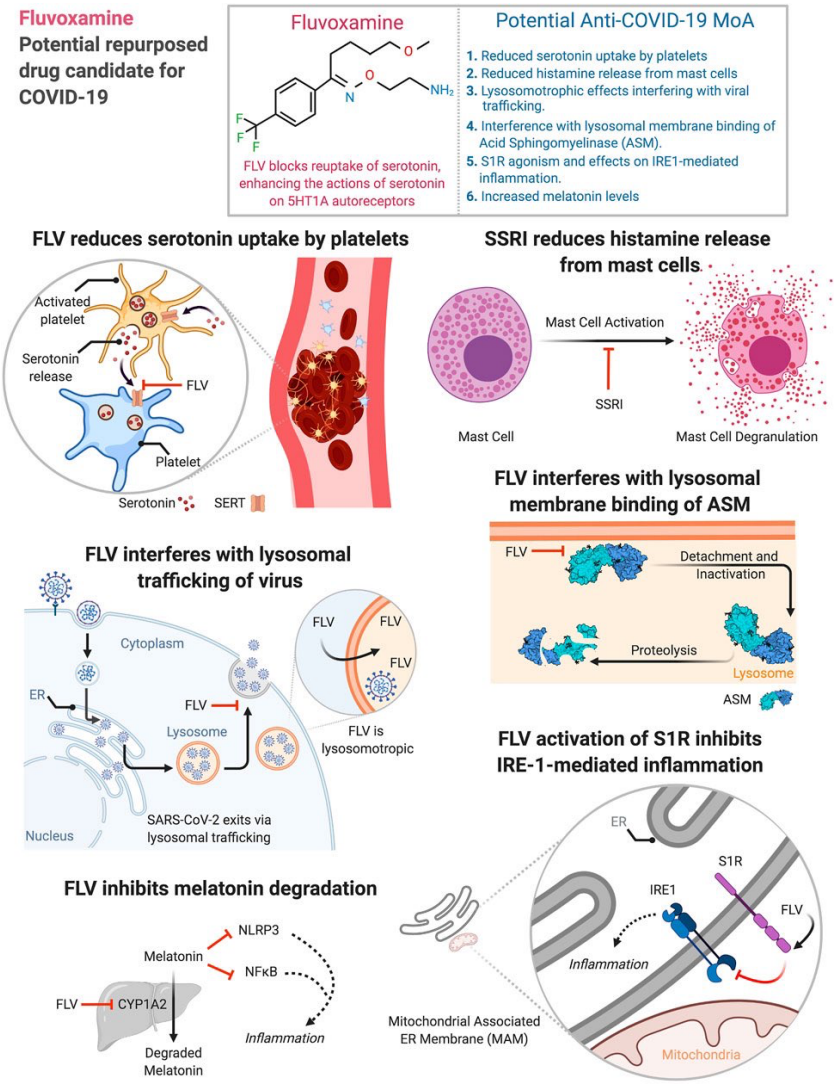


Figure 7 – Potential mechanisms of benefit from the use of fluvoxamine in participants with COVID-19[184] .

1.14.6 Studies of Fluvoxamine in COVID-19

In a preliminary, randomized, double-blind study with a small number of adult outpatients with symptomatic COVID-19 and using an indirect clinical outcome, 80 participants treated with FLV, compared to 72 treated with placebo, had a lower probability of clinical deterioration over 15 days[185] . Participants were randomized within 7 days of symptom onset. These phase II data are striking, as none of the participants treated with fluvoxamine experienced clinical deterioration, whereas 8.3% of participants in the control group experienced such complications.

We tested the hypothesis that fluvoxamine reduces clinical events during the pandemic (TOGETHER Clinical Trial; CAAE 41174620.0.1001.5120). We recruited 1,497 participants, with 741 in the fluvoxamine arm and 756 in the placebo arm. The median age was 50 years (18–102) and the mean time to symptom onset was 3.4 days. The proportion of participants with the study's primary outcomes was lower in the fluvoxamine group compared with the placebo group (79 [11%] of 741 vs. 119 [16%] of 756); relative risk [RR] 0.68; 95% confidence interval in the Bayesian analysis [95% CI]: 0.52–0.88), with a probability of superiority of 99.8%, exceeding the pre-specified superiority threshold of 97.6% (risk difference: 5.0%). Considering the composite clinical outcome, 87% of events were hospitalizations and 12% were emergency department visits and observation > 6 hours associated with SpO2 < 94%. The calculations for the study's primary outcome were similar in the modified intention-to-treat analysis (RR 0.69, 95% CI: 0.53–0.90) and higher in the per-protocol analysis (RR: 0.34; 95% CI: 0.21–0.54). There were 17 deaths in the fluvoxamine group and 25 deaths in the placebo group in the primary intention-to-treat analysis (odds ratio [OR] 0.68; 95% CI: 0.36–1.27). In the per-protocol analysis, considering only participants who adhered to both treatment arms for at least 7 days, we observed 1 death in the fluvoxamine group and 12 deaths in the placebo group (OR 0.09; 95% CI: 0.01–0.47)[186] .

In this same context, we also tested the combination of fluvoxamine and budesonide (100 mg every 12 hours of fluvoxamine combined with 800 mcg every 12 hours of budesonide, both for 10 days) in the TOGETHER clinical trial, involving 1,478 participants with COVID-19, with 738 assigned to the treatment arm and 738 assigned to the placebo arm. There was a significant reduction in primary outcomes (hospitalization and/or days spent in the emergency department) in the treatment arm (1.8 [95% CI – 1.1 – 3.0] vs. 3.7 [95% CI – 2.5 – 5.3], relative risk 0.50 [95% CI 0.25–0.92) with a probability of superiority > 98.7%[187] .

1.14.7 Studies of Fluvoxamine in COVID-19

A retrospective evaluation conducted by the "National COVID Cohort Collaborative Group, National Institutes of Health, USA" assessed the correlation between the use of serotonin reuptake inhibitors (SSRIs) with S1R receptor agonistic activity and the incidence of Long COVID. A total of 17,933 individuals were evaluated, including 14,484 who were not using SSRIs, 1,328 using SSRIs without S1R agonistic activity, and 2,021 participants using SSRIs with S1R agonistic activity. After the necessary statistical adjustments, a 26% reduction in the incidence of Long COVID was observed in participants who were using SSRIs with S1R agonistic activity[188] .

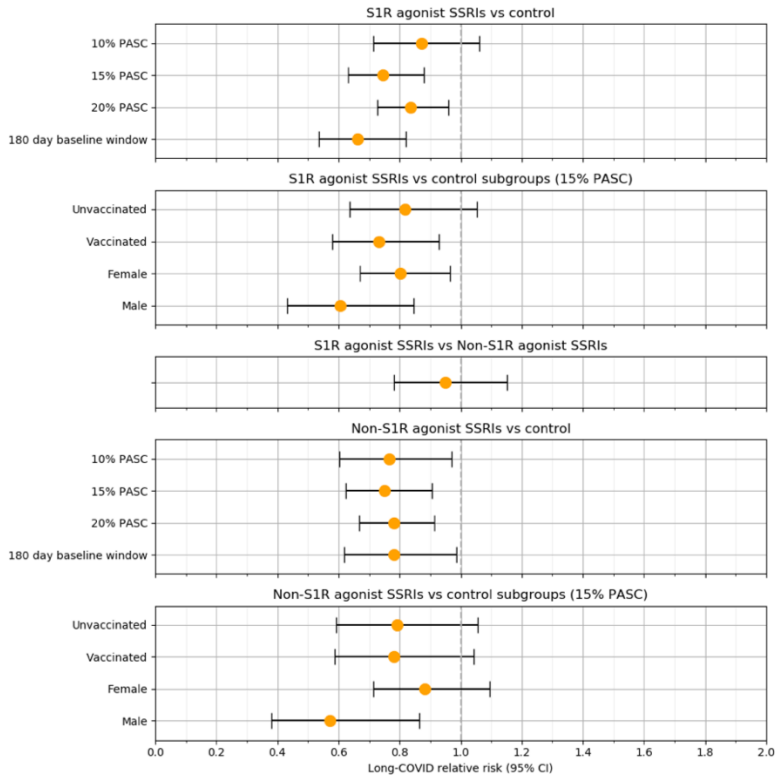
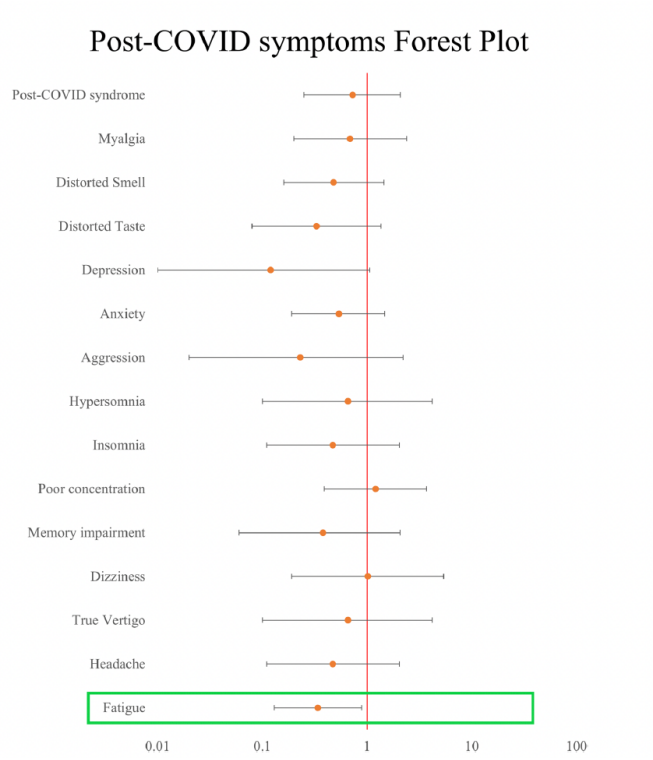


Figure 4. Associations between baseline S1R agonist and non-S1R agonist SSRI exposure and long-COVID for different patient groups and model specifications.

Sidky et al., from reference 188.

Farahani et al. evaluated 100 participants with Long COVID symptoms using 100 mg of fluvoxamine every 12 hours for 3 months in a prospective, randomized, double-blind clinical trial[189] . Various symptoms were assessed. At the end of 3 months, data were obtained from 85 participants (15 were considered lost to follow-up). In the evaluated participants, a 48% reduction in fatigue-related symptoms was identified.



Farahani et al.: data regarding long COVID

1.15 Metformin and COVID-19

Since the emergence of the first cases of viral pneumonia associated with SARS-CoV-2 to the present day, various clinical conditions have been definitively linked to the complications that have occurred, progression of lower respiratory tract infection, respiratory failure, and death. It is believed that these conditions allow the virus to trigger the development of an exacerbated inflammatory response. These clinical conditions are now considered risk factors for severe COVID-19. Among these, advanced age is one of the most important, and associated with it are hypertension, diabetes, coronary artery disease, smoking, and obesity. In this context, obesity stands out, since after adjusting for other risk factors, obesity emerges as a significant factor associated with worsening respiratory status and the need for mechanical ventilation[190] . Participants with a body mass index > 25 $^{kg/m^2}$or men with excess visceral adipose tissue are at high risk of requiring invasive ventilatory support during the course of COVID-19[191] .

1.15.1 Elevated glucose is associated with worse outcomes in COVID-19

Metformin reduces blood glucose levels, but not below physiological levels, by reducing hepatic gluconeogenesis. Poorer glucose control has also been associated with higher mortality and end-organ complications in participants with COVID-19[192] .

1.15.2 Metformin may reduce endothelial damage and its complications.

It has been demonstrated that metformin improves microvascular endothelial function in women, perhaps due to a significant increase in the response to acetylcholine, a decrease in insulin resistance, and a non-significant decrease in tissue plasminogen activator, as previously demonstrated in an 8-week randomized controlled trial of metformin versus placebo in women with angina and normal coronary arteries[193] . Pulmonary vascular endotheliosis was observed in the lungs of participants with COVID-19[194] .[69] Acute endothelial inflammatory changes may be an important mechanism and a therapeutic target in mitigating the sequelae of COVID-19. Metformin reduces thrombosis in long-term follow-up, possibly by inhibiting platelet-activating factor and the release of mitochondrial DNA (mtDNA)[195] , and may be associated with better cardiovascular outcomes through mechanisms beyond glucose control. Coagulopathy and thrombosis associated with COVID-19 are a hallmark of the disease, and the pathophysiology is poorly understood. Furthermore, widespread micro- and macrovascular thrombosis has frequently been reported in autopsies of participants with COVID-19[196] .

1.15.3 Reduction of "neutrophil extracellular traps" (NETs)

Metformin is associated with a significant reduction in neutrophil extracellular traps (NETs) and the neutrophil-to-lymphocyte ratio[197] . NETs are microbiocidal compounds containing DNA, histones, and proteins. Serum from participants with COVID-19 shows elevated levels of these histone and DNA components. It has been hypothesized that excessive NET formation leads to a cytokine storm and microthrombi (possibly independent of tissue factor), culminating in acute respiratory distress syndrome (ARDS) in COVID-19[198] . Lymphopenia and neutrophil infiltration in the pulmonary capillaries have been an important feature of severe COVID-19[199] . Inhibition of NET release by metformin could therefore attenuate the development of downstream lung injury.

1.15.4 Metformin and its immunomodulatory effects

Visceral adipocytes secrete various pro-inflammatory mediators and procoagulant molecules, including interleukin (IL)-6, tumor necrosis factor α (TNF-α), adipokines, and D-dimer;

participants with COVID-19 exhibit elevated production of inflammatory and procoagulant cytokines, which have been identified and associated with the pulmonary inflammatory condition in these participants[200,201] . In participants with type 2 diabetes mellitus, TNF-α and IL-6 are elevated, and IL-10 levels are reduced, with a direct relationship between these changes and the degree of insulin resistance observed in these participants[202] .

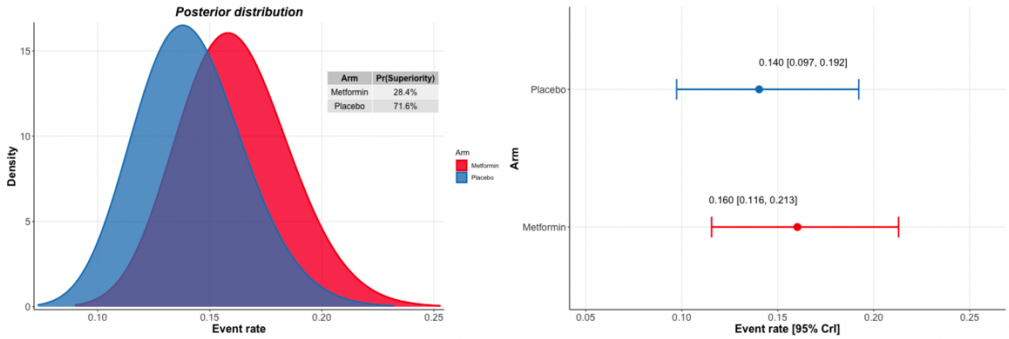
Metformin, a medication for type 2 diabetes, decreases levels of TNF-α, adipokines, and IL-6, and increases levels of IL-10; these changes have been observed in both experimental studies and studies conducted in participants with type 2 diabetes and are more evident in women[203,204,205] . These effects, associated with a reduction in circulating adipokines, may help minimize the degree of inflammatory response and thus reduce the severity of the disease[206] .

The activation of the AMPK/mTor/Stat3 pathway by metformin appears to prevent macrophages from undergoing classic pro-inflammatory activation that produces TNF/IL-6/IL-1β, cytokines that contribute to morbidity in COVID-19[207, 208] . Possible evidence of this effect was observed in a retrospective study by Chen et al., which analyzed 904 participants with COVID-19 and showed that metformin users had lower levels of IL-6 compared to non-users of metformin[209] . Metformin also inhibits toll-like receptor 7 (TLR7) signaling and interferon production, which appears to be important for the pathophysiology of COVID-19[210, 211] . Metformin also inhibits IgE- and aryl hydrocarbon-mediated mast cell activation[212] . Mast cell activation has been implicated as an early indicator of the inflammatory response to SARS-CoV-2 and, possibly, an indicator of an impending cytokine storm. It has been found that mast cells from female rats cause a greater increase in tumor necrosis factor alpha (TNF-alpha) than mast cells from male rats, which may explain the observational findings of reduced mortality in women taking metformin, but not in men taking metformin[213] .

Clinical studies have suggested that clinical complications and mortality in participants with COVID-19 may be lower in those taking metformin; however, the observational and retrospective nature of these studies (medical record analysis), as well as the fact that other studies have not confirmed this association, makes it difficult to adopt metformin as part of the treatment for hospitalized participants[214,215,216,217] . A recent observational study identified metformin as a potential mortality reducer in women[218] .

Our research group evaluated the use of metformin in COVID-19[219] . Between January 18, 2021, and April 3, 2021, we evaluated 418 participants with early-stage COVID-19, with 215

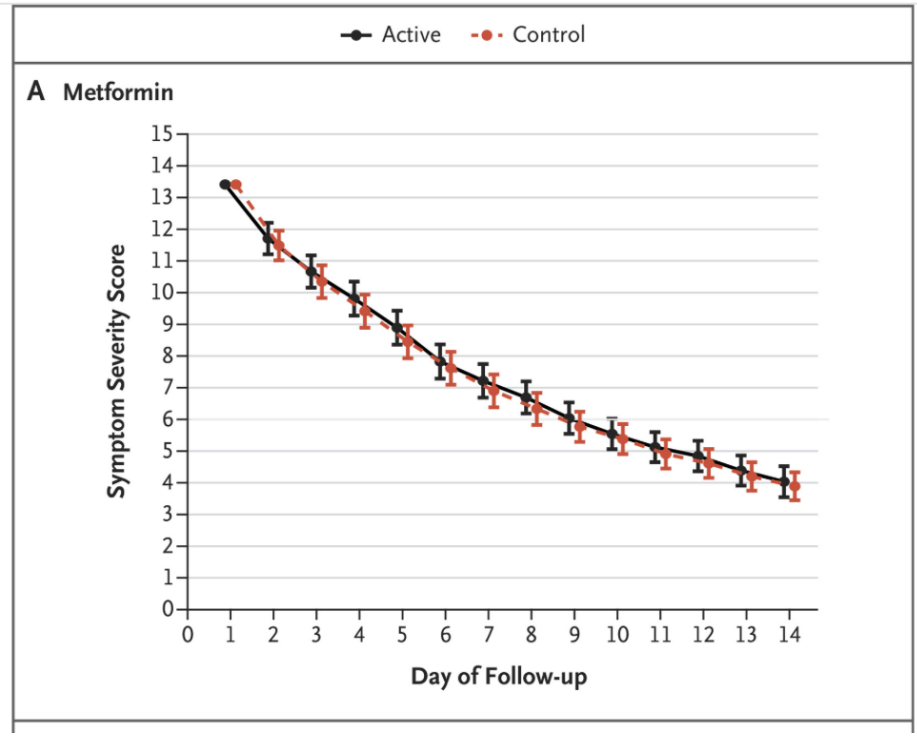
participants randomized to receive metformin (metformin XR 750 mg every 12 hours for 10 days) or a corresponding placebo. Recruitment took place in emergency departments and in dedicated acute respiratory syndrome emergency treatment units. On April 3, 2021, the Independent Data Safety Committee recommended discontinuing this arm due to futility, with a relative risk for primary outcomes of 1.14 (95% CI 0.73–1.81) and a probability of superiority of 0.28.



Intent-to-treat analysis – Metformin in COVID-19, ref 204.

Analysis of data related to symptoms on Day 60 post-randomization indicated a 26% reduction in symptoms of fatigue, dyspnea, and tiredness in the treated group (archived data, unpublished.

Bramante et al. evaluated metformin in COVID-19 in a group of participants with COVID-19 and mild symptoms[220] . Between May 21, 2021, and February 14, 2022, 1,323 participants were recruited into the metformin group, in a factorial design (a subset of participants allocated to the metformin group also received fluvoxamine and ivermectin, with the same rationale applied to the metformin placebo group, resulting in an estimated 33% balance for each drug). Thus, 663 participants were exposed to metformin and 660 participants were not exposed to metformin (considered the placebo-). Recruitment occurred remotely via telephone contact with participants who tested positive for COVID-19. There were no in-person visits, and the primary outcome was clinical improvement on a symptom scale assessed 14 days after randomization. The adjusted rate of the primary outcome was 0.84 (95% confidence interval [CI], 0.66 to 1.09; P=0.19) for participants exposed to metformin, with no statistical significance.

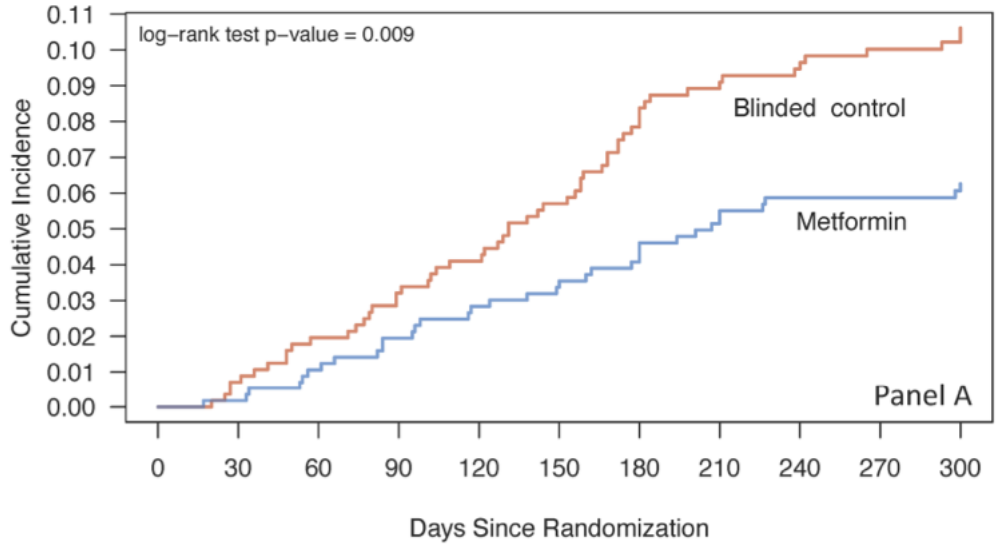


Symptom severity score in COVID-19, ref. 205.

1.16 Metformin and Long COVID

Although clinical trials using metformin in participants with acute COVID-19 have not demonstrated efficacy in reducing clinically relevant events or symptoms, data suggest that prolonged use and/or prolonged follow-up of participants taking metformin may result in a lower incidence of dyspnea, fatigue, tiredness, and nonspecific neurological symptoms.

Bramante et al. reported the results of a 10-month follow-up in their original clinical trial[221] . The authors obtained data from 1,125 participants in a pre- follow-up specified as a secondary analysis of the original study, including 564 participants exposed to metformin and 561 participants not exposed to metformin. Assessments were conducted remotely via a questionnaire sent by email. Among participants randomized to the metformin arm, 6.2% (95% CI 4.2–8.2%) reported worsening symptoms after developing COVID-19, compared with 10.6% (95% CI 8.0–13.1%). The reduction in the risk of developing Long COVID in this population was estimated at 58% compared to the placebo group.



Cumulative incidence of "Long COVID," post-acute sequelae of SARS-CoV-2 infection (PASC), diagnosed by a physician more than 10 months after randomization. Adapted from ref 206.

Although it shows a reduction of over 50% in the incidence rate of Long COVID compared to placebo, the clinical trial by Bramante et al. must be interpreted with caution. This data was collected remotely, without a standardized definition of Long COVID, as this diagnosis was reported by the attending physician at various clinics across the United States. Furthermore, the information was collected remotely, and data were missing for more than 6% of the original sample. The information was based on a proprietary scale, and participants were subsequently exposed to more than one potentially therapeutic agent.

These limitations point to the need to seek evidence regarding a possible link between metformin and long COVID.

2 STUDY OBJECTIVES

The objective of this study is to evaluate the efficacy, safety, and benefits of using fluvoxamine and metformin in participants with a clinical diagnosis of long COVID who are being monitored at Primary Health Care Units in Brazil. The research subject's participation in the protocol lasts up to 90 days, with an initial phase of up to 60 days considered the treatment phase and the period of up to 90 days considered the follow-up period for the study and verification of the study's clinical results.

2.1 Objectives/Primary Outcome

The primary objective is to compare the use of Fluvoxamine and Metformin with placebo for the mean score on the Fatigue Severity Scale (FSS) on day 60 after randomization by at least 1 point.

2.2 Secondary objectives/ outcomes

The secondary objectives are:

To compare fluvoxamine and metformin with placebo for the mean FFS score 30 days after randomization.

To compare fluvoxamine and metformin with placebo for the mean FSS score 60 days after randomization.

To compare fluvoxamine and metformin with placebo regarding the mean difference in the change in health-related quality of life (HRQL) as measured by the EQ-5D-5L at 60 days from randomization.

Compare fluvoxamine and metformin with placebo for the odds ratio of all-cause mortality at 60 days after randomization.

Compare fluvoxamine plus metformin with placebo for the odds ratio of unexpected hospitalization from all causes at 60 days.

To assess the safety of fluvoxamine and metformin in this population of participants.

To evaluate the mid-term post-study effects (90 and 180 days) of the investigational medicinal products on the chronic fatigue scale (FSS scale) and quality of life (EQ-5D-5L).

3 RESEARCH PLAN

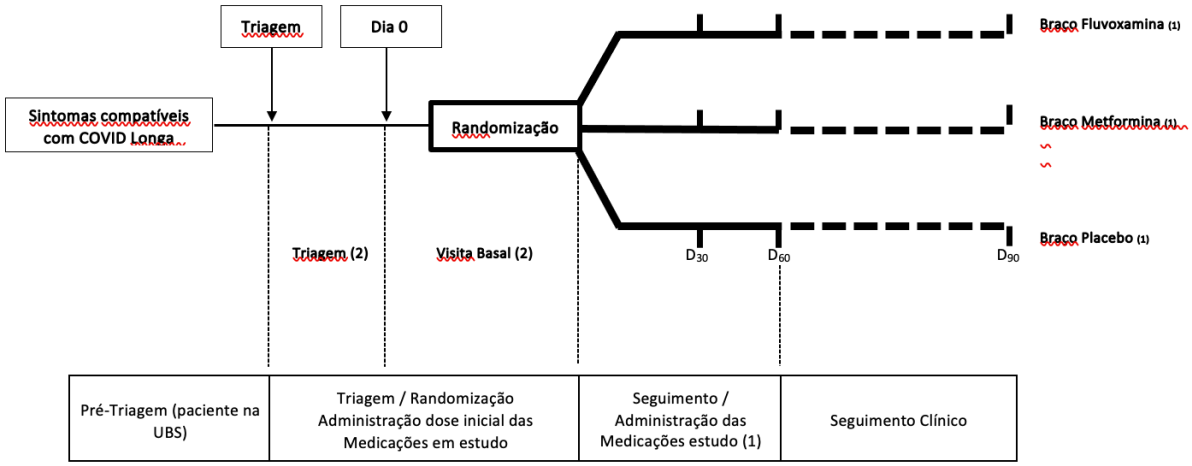
3.1 Study Design

This is a prospective, multicenter, adaptive, double-blind, randomized, placebo-controlled study to evaluate the effect of different therapies on reducing fatigue levels in participants with long-standing chronic fatigue syndrome. The treatment groups will be fluvoxamine, metformin, and the corresponding placebo.

Participants will be randomized to one of the study arms using an independent, automated, iterative centralized randomization system (IVRS or IWRS). The protocol includes an adaptive phase to accommodate any pre-specified modification needs.

The protocol is designed to enroll 1,500 participants, with 500 participants in each study arm in an equal randomization ratio (e.g., 1:1:1). The protocol includes three planned interim analyses to occur at 25%, 50%, and 75% of the total number of participants. These interim analyses will allow for potential termination based on superiority, in the event of clear evidence of efficacy, or termination due to futility, in the event of insufficient evidence of efficacy. The decision thresholds for efficacy and futility are calibrated using statistical simulations to ensure control of the Type I error rate at 0.025 in each of the pairwise comparisons between the experimental treatment arm and the control. These interim evaluations will be conducted by the Data Safety Committee, which will be supported by statisticians, and decisions will be communicated to the Steering Committee for appropriate regulatory action, when applicable. Any decision to discontinue treatment will be subject to immediate notification to regulatory authorities and the Ministry of Health, in accordance with current regulations.

Fluxograma da Pesquisa
Braços Fluvoxamina, Metformina e Placebo



1. Tratamento: Fluvoxamina, Metformina e placebo em grupos paralelos pelo período planejado. Posologia das medicações varia conforme a alocação do paciente em um braço do estudo (Para cada braço há o correspondente placebo, na mesma formulação e posologia. Medicações serão interrompidas a qualquer momento se houver evidência de reação adversa, a critério do sujeito da pesquisa ou por recomendação do DSMB (eficácia, futilidade ou segurança do participante).
2. Triagem e Randomização devem ser realizadas na mesma visita. Assegurar que o paciente seja randomizado por ocasião do atendimento.
3. As visitas subsequentes: D_{30} a D_{60}, D_{90} serão realizadas através de contato telefônico, telemedicina ou aplicativos de mídias sociais, entretanto com possibilidade de visitas presenciais, caso necessário. Em qualquer momento visitas extras de segurança poderão ser realizadas, inclusive presenciais. A visita D_{60} é considerada a visita de desfecho para a pesquisa. A visita D_{90} é considerada visita pós-estudo, para acompanhamento de eventuais complicações tardias/ persistência de sintomas relacionados ao COVID-19 e também para avaliação eventual de reações adversas tardias aos medicamentos da pesquisa. Esta será realizada através de contato telefônico. Não há previsão de visitas presenciais regulares nesta pesquisa, entretanto recomendamos que a visita D_{60} seja preferencialmente realizada presencialmente.
4. Visitas adicionais poderão ser realizadas seja por sugestão da equipe de pesquisa seja por solicitação do participante.

Research flowchart

3.2 Rationale for the study design

The COVID-19 pandemic has not only emphasized the importance of randomized clinical trials following proper methodology and adopting consistent practices in the planning, conduct, and analysis of the data obtained, but above all has demonstrated the need for the development and conduct of large-scale clinical trials, structured according to a master protocol, in a coordinated manner and through global collaboration[222] . Therapeutic research for acute COVID-19 has resulted in futile efforts by most research groups, as, although thousands of trial records have emerged, most of them lack a consistent methodology, exhibit significant biases, and contain major flaws in their conduct and in the interpretation of results.

Based on this perspective for long COVID-19, we proposed an adaptive platform study project, similar to the TOGETHER study conducted by our research group in several municipalities in the state of Minas Gerais and approved by this committee. Protocols with an adaptive design, through the “perpetual” study of more than one therapeutic intervention, can be evaluated and discarded (via a robust statistical analysis and evaluation plan) if they show no evidence of benefit[223] . These studies create opportunities to generate more efficient knowledge, which can be

incorporated into routine clinical practice and promote continuous improvements. With their common platform and infrastructure, efficient use of control arms, and ability to expedite the launch of new study interventions, adaptive clinical trials can offer numerous advantages in identifying effective drugs and devices to combat COVID-19 and in comparative efficacy scenarios—quickly and efficiently, without compromising the quality of a randomized clinical trial[224,225(,) 226] .

3.3 Rationale for the dose/regimen, route of administration, and duration of treatment

3.3.1 Fluvoxamine

Fluvoxamine is an SSRI antidepressant with anti-inflammatory activity due to its S1R agonist effect. In the TOGETHER clinical trial, we administered a dose of 100 mg orally every 12 hours for 10 consecutive days in patients with acute influenza-like illness. We used this dose in two arms: initially as monotherapy[186] and subsequently in combination with budesonide[187] , with tolerability and safety comparable to the placebo group. A dose of 50 mg administered orally every 12 hours was tested in some clinical trials but did not demonstrate efficacy, suggesting that a 50 mg dose every 12 hours is insufficient to elicit anti-inflammatory effects via S1R receptors[220,221] .

The 60-day period is necessary because evidence suggests that Long COVID is related to a severe and persistent disruption of the immune-inflammatory balance, involving key pathways that require at least 3–4 weeks to restore equilibrium. Furthermore, clinical case reports using these drugs for a short period of time have failed to demonstrate benefits.

3.3.2 Metformin

Participants randomized to the metformin arm will receive a dose of 500 mg twice daily for a period of 60 days. We chose to modify the metformin dose in the clinical trial for two reasons: (1) To align with the international literature, which has used a 500 mg dose every 12 hours in clinical trials to evaluate Long COVID, and (2) following a blinded analysis of the first 100 recruited participants, where we identified a dropout rate due to mild adverse effects (diarrhea, abdominal pain, nausea) in 9% of participants (common symptoms associated with the use of higher doses of metformin). In particular, the study by Bramante et al. observed a lower incidence of Long COVID in participants in the COVID OUT study's Metformin Arm, where the dose used was 500 mg every 12 hours[218] . There is evidence that doses exceeding 750 mg twice daily do not result in a better inflammatory profile. The metformin dose was selected based on previous observational evidence of a dose response at

1,000 mg per day and is consistent with our previous TOGETHER study of metformin vs. placebo[219] and a similar study[220] . We chose to use the extended-release formulation because it causes fewer gastrointestinal side effects and has more consistent and stable bioavailability, which is an advantage in providing a uniform serum dose. Since metformin is well tolerated over long periods[227] , to observe its potential effect in participants with prolonged COVID-19, we chose a 60-day duration, considering the pathogenesis of the immuno-inflammatory changes present in Long COVID, which often require a therapeutic action period of 3–4 weeks for the expected effects, this being a posspossible explanation for the futility of using metformin in acute forms of COVID-19.

3.5 Rationale for the trial

Currently, there are no approved therapies for the treatment of Long COVID. Given the complex disease process, high work disability, and persistent symptoms associated with the condition, evidence is needed to support the safety and efficacy of treatments.

Few randomized studies of outpatient treatment for COVID-19 have followed participants for a long period after acute treatment to assess the effect of early treatments on the incidence of post-COVID-19 conditions. Although the mechanism of action has not been fully elucidated, metformin is known to alter glucose metabolism and is being investigated as an antiviral agent, demonstrating in vitro and in vivo activity against SARS-CoV-2. Recent studies suggest that metformin may also serve as a therapeutic option for participants with long COVID-19[221] .

4 RESEARCH PLAN

4.1 Study Design

The study consists of an in-person screening and randomization visit, which will occur simultaneously, and visits conducted via in-person visits, telephone contact, and social media applications using video conferencing. The following visit schedule will be implemented for the metformin and corresponding placebo arms (adjusted for treatment days according to the intervention arms):

V1 (Day 1) - Screening, Baseline, Randomization (Start of treatment phase)
V2 (Day 7) - Follow-up Visit / Tolerance to Investigational Medicinal Products
V3 (Day 30) - Follow-up visit
V4 (Day 60) - Follow-up visit (in-person visit recommended)
V5 (Day 90) - Follow-up visit after completion of the investigational product
V6 (Day 180) - Follow-up visit after completion of the investigational product

The D90 and D180 visits are considered post-study visits. The objective of these visits is to evaluate late effects of the investigational medical products and the persistence of any therapeutic effect observed during the treatment phase. This phase is an open-label, post-study phase.

4.1.1 Visit V1: Screening visit/baseline visit/randomization General study design

Screening and the baseline visit (randomization) (Day 1) will take place in person at the participating research center. After providing informed consent for the screening process, potential participants will be asked to complete the FSS questionnaire. The questionnaire will be scored to determine whether the potential participant meets the moderate fatigue threshold. If the participant meets this threshold, as well as all other inclusion criteria and none of the exclusion criteria, they will be invited to participate in the study.

After completing the above screening activities, the research team will obtain the participant's informed consent to participate in the study. Participants who do not provide informed consent will be considered screening dropouts.

The following items will be collected at the baseline visit: participant demographics, medical history, comorbidities, risk factors, concomitant medications, physical examination findings, vital signs (including temperature, heart rate, respiratory rate, and oxygen saturation in room air), and details of the participant's COVID-19 medical history.

4.1.2 Treatment Phase (Randomization)

After all procedures for the baseline visit have been completed, all inclusion criteria have been verified, and it has been determined that the patient does not meet any exclusion criteria for the study, participants will be considered eligible for the treatment phase and then randomized to fluvoxamine, metformin, or placebo in a 1:1:1 ratio.

This randomization process will be conducted centrally or via the IWRS system, and treatment kits identified by random numbers will be allocated. The kits will be provided in a manner that prevents any individual from identifying the study drug. Participants will begin the assigned treatment (fluvoxamine, metformin, or placebo).

4.1.3 Post-treatment phase (post-study follow-up)

This phase aims to monitor the medium-term effects of using the investigational medical products, with a primary focus on the chronic fatigue scale (FSS scale) and quality of life (EQ-5D-5L scale).

4.1.4 Duration of Participation in the Study

Participation for each eligible study participant includes a screening visit (Day 1), followed by the 60-day treatment phase. A post-treatment visit will be conducted 30 days after the end of the treatment phase. The first day of medication administration is at the time of randomization and before the participant is discharged from the facility where they were enrolled in the study. The study will continue into a follow-up phase after completion of the investigational product, with follow-up visits on Day 30 and Day 60, with the final visit occurring 90 days after the randomization date (30-day post-investigational product visit). Results will be evaluated on Days 30, 60, and 90 after randomization.

A safety assessment visit will be conducted on Day 7 post-randomization to assess the tolerability of the interventions and check for possible adverse events or adverse reactions to the investigational medical product under evaluation.

Participants who discontinue the investigational product prematurely will be invited to remain in the study to complete the remaining procedures and will receive standard care for the long-term treatment and follow-up of COVID-19.

5 SELECTION AND WITHDRAWAL OF PARTICIPANTS

5.1 Number of Participants

For detailed information on the rationale for the sample size, see Section 12.

5.2 Inclusion Criteria

Participants must meet all of the following criteria to be eligible for participation in this study:

1. Age 18 years or older at the time of screening.
2. Willing and able to provide written informed consent, or have a legal representative who can provide informed consent (where approved locally and nationally).
3. A previous confirmed case of SARS-CoV-2 infection (e.g., reports having had a positive nucleic acid amplification test or a positive professional-use or self-test SARS-CoV-2 rapid antigen test).
4. Participants with clinical symptoms consistent with LONG COVID according to international definitions: (www.nice.org.uk/guidance/ng188 , https://www.who.int/publications/i/item/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1), and fatigue symptoms with an average score of at least 03 on the Fatigue Severity Scale (FSS);
5. Have a diagnosis of the most recent episode of COVID-19 within 24 months of the screening visit date.
6. Not currently hospitalized or in need of hospitalization, or having been hospitalized in an intensive care unit during the COVID-19 episode.
7. Participants with the following vital signs:
    a. Heart rate between 55 and 100 bpm;
    b. Temperature below 38°C;
    c. Oxygen saturation ≥ 95%.
8. Participants who are capable of becoming pregnant or whose partners are capable of becoming pregnant must agree to use appropriate contraceptive methods during the study and for up to 90 days of follow-up.
9. Symptoms of fatigue cannot be attributed to another cause (in the researcher's opinion).
10. Willingness to follow all study procedures.

5.3 Exclusion Criteria

Participants who meet any of the following criteria will not be eligible to participate in the study:

1. Known acute SARS-CoV-2 infection;
2. Inability to understand the content of the Informed Consent Form or to follow the study procedures;
3. Known prior diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome, unrelated to SARS-CoV-2 infection;
4. Known pre-existing dysautonomia, unrelated to SARS-CoV-2 infection;
5. Diabetes mellitus (exclusion criterion for the Metformin and Metformin Placebo arms);
6. Known history of creatinine clearance below 30 ml/min or on dialysis;
7. Known stroke within the 3 months prior to screening;
8. Known severe anemia, defined as < 8 g/dL;
9. Body Mass Index (BMI) > 40.
10. Known diagnosis of Lyme disease;
11. Any use of illicit drugs other than marijuana within 30 days prior to informed consent;
12. Pregnant women or women of childbearing age who do not agree to use an effective method of contraception for 90 days from the date of signing this consent form;
13. Women who are breastfeeding;
14. Expected hospitalization for elective surgical procedures lasting more than 48 hours;
15. Contraindications for the METFORMIN arm
    a. Participants currently taking metformin;
    b. Participants with creatinine clearance < 30 ml/min, a history of KIDIGO IV, or currently undergoing dialysis.
16. Contraindications for the FLUVOXAMINE arm
    a. Use of serotonin reuptake inhibitors (donepezil, fluoxetine, escitalopram, paroxetine);
17. Current severe comorbid psychiatric disorder (e.g., clinical depression, anxiety, sleep disorder, eating disorder, substance abuse) that is uncontrolled and associated with significant symptoms or requiring the use of a medication contraindicated in this study;
18. Clinical history of moderate to severe hepatic impairment or hepatic cirrhosis with a Child-Pugh score of C or higher;
19. A clinical history of severe chronic lung disease with significant limitation of activities;
20. Inability of the participant to provide informed consent or adhere to the procedures proposed in the study;
21. Taking medications known to cause chronic fatigue as a side effect (except for participants with chronic cardiovascular disease who are on chronic beta-blocker therapy);
22. Known hypersensitivity and/or intolerance to fluvoxamine or metformin;

23. Any clinical condition that, in the investigator's opinion, may preclude participation in this study.

5.4 Inclusion Criteria

Participants may be randomized if they meet the inclusion criteria and do not meet any exclusion criteria for the study.

5.5 Discontinuation of the investigational product or removal of participants

5.5.1 Discontinuation of the investig

During the treatment phase of the study, the participant may discontinue the investigational product at any time and at their discretion. Similarly, the investigator may discontinue the investigational product whenever deemed necessary, whether due to an adverse event or to ensure patient safety.

Participants who discontinue treatment with the investigational drug without apparent justification after randomization and before the conclusion of the study will be encouraged to resume the medication and continue in the study as usual. If the medication is discontinued, the participant will be offered the option to remain in the study for the collection of outcomes. These participants will be treated according to the standard of care determined by the investigator.

5.5.2 Withdrawal from the Study

5.5.2.1 Withdrawal of Informed Consent

Within the provisions of informed consent and clinical judgment regarding participant safety, every effort should be made to ensure that participants complete the treatment phase and post-treatment visits. Participants will be informed that they may withdraw from the study at any time. However, if a participant withdraws from the study, every effort will be made to determine the reasons why the participant withdrew their consent. Although participants are not required to provide a reason for withdrawing consent, the investigator will make every effort to understand these reasons, fully respecting the participant's rights. The reasons for withdrawal of consent, when provided by the

participant, will be recorded in the medical record, and the center will do everything possible to ensure that the participant completes the early termination procedures described. Every effort will be made to contact a participant who fails to attend and/or does not attend a study visit by telephone to ensure that the participant is in satisfactory health.

A participant who wishes to withdraw consent for the use of investigational medical products may agree to:

Provide information about their own health status by phone or other means until the date of the final study visit.

Allow family physicians or family members to be contacted to provide information about the participant's health status

Allow for a final contact at the end of the study

5.5.2.2 Participant suspended by the investigator of the Study

The investigator and the designated team may use their medical judgment to terminate a participant's enrollment in the study if they determine that the participant's continued participation in the study could result in safety risks to the participant. The investigator must immediately inform the study's medical monitor of plans for the early withdrawal of a participant from the study. Participants withdrawn by the investigators will also have the opportunity to consent to the three options described above. All participants withdrawn early from the study for any reason must complete the Early Study Termination procedures described and be monitored for safety after receiving the last dose of study medication. Randomized participants who are withdrawn from the study for any reason will not be replaced.

5.5.2.3 Participants with early discontinuation

For all participants who drop out or discontinue the study prematurely (including participants who withdraw their consent), survival information may be verified through a search of a public database at the end of the study.

6. STUDY TREATMENTS

6.1 Treatment blinding

To minimize the potential for bias during the treatment phase, treatment randomization information will be kept confidential by a non-blinded biostatistician and will not be disclosed to third parties until the study database is finalized for statistical analysis upon completion of recruitment and follow-up of the last scheduled patient. The study is blinded, and neither the patient nor the investigator, the research team, nor existing committees will have access to the contents of the vials, which are produced sealed and hermetically closed. Similarly, the sponsor and the designated person will not have access to the randomization data. Treatment vials will be distributed using codes maintained by an unblinded biostatistician not involved in the research. The Data Monitoring Committee (DMC) and the drug safety team will not have access to participant allocation during the

interim assessments planned in the study, except in foreseeable situations (decision to discontinue any arm of the study, whether due to efficacy or futility, termination of the study, or for reasons of overall participant safety).

The clinical trial supply management team will have access to general information on investigational products at the site level for the management of packaging and distribution activities, as well as for the supervision of investigational product inventory levels in drug warehouses and at study sites, but will not have access to the allocation codes for these products.

6.2 Dosage Form/Administration

Metformin will be provided to the participant in the form of 500 mg extended-release tablets for oral use.

Fluvoxamine will be provided to the participant in the form of 100 mg tablets for oral use.

6.3 Dosage and administration

Participants assigned to the 500 mg extended-release metformin treatment group should take the medication every 12 hours for 60 days.

Participants assigned to the 100 mg dose of fluvoxamine should take the medication every 12 hours for 60 days.

6.4 Supply of the investigational medicinal product

The investigational products will be provided to the participant at no cost, with instructions for use solely for the purpose of the research. Bottles of identical format will be provided, containing sufficient medication for use as scheduled. The patient must return the blister packs for accounting of the medications delivered.

The medications used in the study will come from pharmaceutical manufacturers with commercial authorization for their production, already approved by ANVISA, or through imports authorized by ANVISA for specific use in this research protocol.

6.5 Study Treatment Allocation

Each eligible participant will be assigned to one of three treatment groups via an Internet-based remote randomization system (IWRS), namely: metformin, fluvoxamine, or the corresponding placebo.

After enrollment in the initial phase of the study, each participant will receive instructions on the appropriate dosage of the medications and individualized instructions on when to take them and other concomitant medications, after considering the participant's current medication regimen. The participant will be instructed to follow the agreed-upon dosing instructions for the remainder of the study to encourage adherence. The investigator will determine whether the study medication administration instructions need to be changed at each scheduled telephone follow-up visit, and any changes will be communicated to the participant.

Participants who qualify for the treatment phase will be randomized to receive the study products as allocated to one of the study arms.

Participants will also be instructed to keep their empty/unused medication bottles, which will be collected by the research team on $_{\text{Day 60}}$for assessment of compliance during the treatment phase. Participants will be instructed to return empty/unused medication blister packs in the containers in which they were originally provided.

Adherence will be documented. Adherence will be assessed based on the number of prescribed medications, the duration of treatment, and the quantity of dispensed and returned medications (used and unused). Adherence as reported by the study participant will also be considered.

Treatment adherence will be considered satisfactory if the participant has taken 80% of the prescribed dose for the study period.

6.6 Delivery , storage, and accounting by the research center

6.6.1 Delivery by the study site

Once the study site has been approved to receive the study drug, it will receive an initial shipment of investigational medicinal products sufficient to treat 20 participants. The need for replenishment of the study drug will be assessed regularly, taking into account the number of enrolled participants, the number of participants currently being treated at the study site, and overall study participation.

6.6.2 Storage

The pharmacist or their representative will verify and confirm receipt of each shipment of medication. The medications will be shipped and stored at room temperature not exceeding 30°C and away from direct sunlight. All study medications will be stored in a secure location. No participants other than those included in this specific clinical trial should take the medications provided for this study. The medication provided for this study must not be used in any animal or laboratory research.

6.6.3 Accounting

All investigational products dispensed to participants must be accurately recorded in the investigational product inventory maintained at the study site by the study pharmacist or qualified representative. Participants must be instructed to return all investigational products dispensed to them (blister packs and containers, whether used or unused), which will be collected by the research team during follow-up visits. All used investigational product blister packs and containers will be retained on-site by the study pharmacist or qualified representative for verification by the study monitor. Verification of inventory and adherence to the investigational product for all investigat s will be performed by the study pharmacist or qualified representative at each scheduled study visit.

6.7 Change in medication dose

6.7.1 Adverse reactions during use of investigational products

The study participant must contact the study team if they experience any adverse reaction they believe is associated with the investigational product. Similarly, the patient will be monitored daily via safety phone calls to check for the presence of unwanted symptoms, adverse reactions, and other signs or symptoms that may be present. The participant may be scheduled for an additional safety visit whenever the investigator deems it necessary, based on the information obtained during the telephone contact.

The decision to temporarily discontinue the medication may be made at any time by the patient or the investigator. An attempt should be made to resume the investigational products whenever possible.

6.7.2 Standard of Care

During the treatment phase, all participants will receive standard of care in accordance with the recommendations of the guidelines.

6.8 Prohibited therapy, special considerations, and concomitant treatment

6.8.1 Prohibited medications

During the study, the following medications will be prohibited while the patient is being treated with the study medications:

Fluvoxamine Arm: Selective serotonin reuptake inhibitors (except sertraline)

1. Metformin Arm: Metformin

6.8.2 Concomitant medications

Information regarding concomitant medications (prescription medications, over-the-counter medications, herbal and naturopathic medications, etc.) will be collected at the start of screening and at each follow-up visit.

In general, participants should continue with the same medications and regimens they were taking at the time of study enrollment. The doses of these concomitant medications should be kept as stable as possible during the study. In general, medications that the investigator considers appropriate for the treatment of any intercurrent illness or preexisting condition that is not on the list of prohibited medications or that does not constitute an exclusion criterion for participation in this study will be permitted.

7. RISKS AND PRECAUTIONS

7.1 Precautions

The investigator must be aware of the administration of investigational drugs in the following situations:

- Depression or psychiatric conditions: These participants must be carefully evaluated, and participation may be permitted if there is no evidence of uncontrolled symptoms, worsening, or major depression. Participants with severe psychiatric conditions should not participate in this research program.
- Participants should eat after taking the medications. It is not recommended to take them on an empty stomach or to fast immediately after taking the medications.
- Participants with a history of seizures may participate if they have not occurred in the past 60 days and are stable and under pharmacological control.

7.2 Adverse Reactions

7.2.1 Fluvoxamine

Most adverse reactions reported in clinical studies conducted with fluvoxamine are gastrointestinal symptoms, generally of mild intensity (nausea, dyspepsia, mild diarrhea, abdominal pain). Other adverse reactions: agitation, anxiety, insomnia, headache, anorexia, palpitations, hyperhidrosis, malaise, QT interval prolongation (QTcF ≥ 450 msec). Aside from gastrointestinal symptoms, the occurrence of other symptoms is uncommon in treatments lasting less than 30 days.

7.2.2 Metformin

Most adverse reactions reported in clinical studies conducted with metformin are self-limiting and resolve upon discontinuation of the medication or with non-pharmacological measures. Common reactions to metformin use include: heartburn, stomach pain, nausea or vomiting, bloating, gas, diarrhea, constipation, weight loss, headache, and an unpleasant metallic taste in the mouth.

8. STUDY PROCEDURES

8.1 Screening Procedures

Before any specific study procedure, the participant will receive an explanation of all study procedures and must date and sign an informed consent form (ICF) approved by a Research Ethics Committee (REC). The screening visit will be conducted (1) at Primary Health Care Units / Outpatient Clinics.

Eligible participants will be identified during the clinical consultation. Participants diagnosed with long COVID-19 will be invited to learn about the research project. If they express interest, they will be referred to a designated and trained research team member who will present the proposed research program and provide the Informed Consent Form (ICF), which will be presented in accordance with current regulatory standards for clinical research. Research procedures will only begin if participants express interest in participating in the research program and sign the ICF. During the screening visit, participants will receive a unique participant number, which will be generated during the registration of the screening visit in the IWRS.

Participants are first screened to identify those who meet the eligibility criteria. When a participant meets all eligibility criteria, they begin the baseline visit phase.

The participant signs the TCLE
Review of eligibility criteria.
Pregnancy test for women of childbearing age.
Completion and scoring of the FSS.
Other procedures scheduled for the visit.

8.2 Day-1 procedures ation / randomization

The procedures performed prior to administration of the investigational drug and randomization must be performed immediately after confirmation of eligibility criteria and obtaining informed consent for participation in the study. The following procedures will be performed during this visit:

- Registration in the IWRS.
- Participant demographic data.

- Medical history, including elective surgical procedures scheduled within 60 days of randomization;
- Comorbidities and risk factors.
- Concomitant medications.
- Physical examination findings.
- Vital signs (including temperature, heart rate, respiratory rate, and oxygen saturation in room air).
- Details of your medical history of COVID-19.
- Randomization and administration of the first dose of the investigational product/placebo.
- Dispensing of the investigational product/placebo and instructions regarding it.
- Instructions regarding subsequent visits.

  Participants will receive their first dose of the investigational medical product (fluvoxamine, metformin, or corresponding placebo) at the research center before being discharged home. They will be instructed to take the medications twice daily for 60 days.

8.3 Procedures during participant follow-up

In-person follow-up visits will take place on days 7, 30, 60, 90, and 180, all at intervals of ± 5 days, following randomization. Participants will be asked to complete the FSS and the EQ-5D-5L 30 days, 60 days, and 90 days after randomization. Additionally, on days 7, 30, 60, and 90, the local research team will verify the participant's status regarding the outcomes of all-cause mortality and unplanned hospitalization for all causes. The research team will collect data on adherence to the experimental product and the placebo (Days 7, 30, and 60). The research team will also document concomitant medications. Participants will be monitored for adverse events and laboratory abnormalities (if they have undergone tests) at each visit.

During the post-study period (visits on days 90 and 180 after randomization), symptoms present at the Day 60 visit will be recorded, as well as new events and symptoms. The D90 and D180 questionnaires (FSS Scale and EQ-5D-5L) will be administered.

The above data will be collected directly from the participant, from the participant's medical record, or from the healthcare professional(s) responsible for treatment if it is impossible to collect the data directly from the participant. If a participant is unable to return to the health center for a follow-up visit, the research team may contact them and conduct the visit via telephone, social media, and/or telemedicine. Data may also be collected through valid electronic communication methods.

8.4 Procedures for Unscheduled Visits

An unscheduled visit may occur at the researcher's discretion or due to the patient's need and may take place during the treatment period up to the final study visit. During an unscheduled visit at any stage of the study, the following activities will be performed if conducted via telephone:

- Assessment of the reason for the unscheduled visit and determination of the course of action.
- Assessment of adverse events.
- Documentation of concomitant medications.
- Adherence to the investigational product/placebo.

Any other study assessments may be performed at the investigator's discretion during an unscheduled visit. In the event of clinical progression of complications expected for long COVID-19, related adverse events will be considered as expected for the clinical condition presented.

8.5 Procedures for early discontinuation of treatment/study withdrawal

For participants who withdraw prematurely from the study (before the Day 90 visit), the center must make every effort to ensure that the participant completes the follow-up visit, which must be conducted on the day of withdrawal or as soon as possible after withdrawal. The assessments conducted at the end-of-study visit must be the same as those for the Day 90 visit.

Participation in the study implies agreement to the proposed post-study period, which consists of two visits (conducted via telephone, social media, or telemedicine), one to be conducted on Day_{90}(30 days) and the other on Day_{180}(120 days) after the end of the therapeutic intervention phase.

9 STUDY ASSESSMENTS AND PROCEDURES

9.1 Laboratory Tests

In women of childbearing age, a pregnancy test is planned, and the biological material to be used is urine or serum. Laboratory tests may be performed to investigate adverse events or changes for which the researcher deems laboratory evaluations necessary, always with the participant's safety and well-being in mind.

9.2 Vital signs

The following vital signs data will be collected:

Temperature

Heart rate

Respiratory rate

Arterial oxygen saturation using a digital oximeter.

Weight and height (reported by the patient)

Respiratory rate will be measured by digital oximetry or physical examination (vital signs).

9.3 Physical Examination

The researcher will perform a brief physical assessment of the participant and document relevant findings in the participants' medical records.

9.4 -reported outcomes

The patient-reported outcome questionnaires (FSS and EQ-5D-5L) will be completed by participants at each visit, always as the first activity of the visit. Study coordinators will review the participant's responses immediately after the participant completes the questionnaires to ensure that all questions are answered.

The FSS questionnaire is a validated 9-item scale that measures the severity of fatigue and its effect on an individual's activities and lifestyle, using a 7-point Likert scale (1 = strongly disagree and 7 = strongly agree). The questionnaire asks the individual to rate the severity of fatigue over the past week. A low score indicates that the individual disagrees with the statement, and a high score indicates that the individual agrees with the statement. The response options selected for each of the 9 statements are summed, and the sum is divided by 9 (the number of questions) to obtain the

final mean score. The minimum average score is 1, and the maximum average score is 7. A score below 4 indicates that participants are not experiencing clinically relevant fatigue, which is considered an exclusion criterion for participation in the study. The Fatigue Severity Scale is commonly used in participants diagnosed with arthritis, fibromyalgia, multiple sclerosis, chronic fatigue syndrome, Parkinson's disease, stroke, and COVID-19. The validity and reliability of the questionnaire have been evaluated in different populations, including those studied following COVID-19.

The EQ-5D-5L is used globally as a generic measure of health status. The EQ-5D-5L descriptive system includes five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. Participants are also asked about their health status by checking the box next to the most appropriate statement in each of the five dimensions. This results in a single-digit number that expresses the selected level for that dimension. The digits from the five dimensions can be combined into a 5-digit number that describes the patient's health status. The EQ visual analog scale records the patient's self-assessment of health on a vertical visual analog scale, where the endpoints are labeled "The best health you can imagine" and "The worst health you can imagine." This can be used as a quantitative measure of health outcomes that reflect the patient's own judgment. The EQ-5D-5L is widely validated, and its applicability is recommended by regulatory agencies (FDA, EMA, and ANVISA).

9.5 Collection of Additional Data

The research team will also collect data on adherence to the investigational medical product. The research team will also document concomitant medications. Participants will be monitored for adverse events and laboratory abnormalities in tests performed for other purposes at each visit.

9.6 Contraception in women of childbearing potential

For women of childbearing potential, a urine or serum pregnancy test will be performed at the screening visit. A pregnancy test will be performed on all women of childbearing potential (childbearing potential being defined in this protocol as at least one menstrual period occurring in the past 12 months in women aged 18 to 55 years). Any pregnancy s during the treatment phase of the study will be monitored until delivery to assess potential complications and adverse events.

10 EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

10.1 Definition of Adverse Events

[228]An adverse event is any unfavorable medical occurrence experienced by a patient or clinical trial participant who has received a drug and that does not necessarily have a causal relationship with that treatment. Therefore, an ADE may be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or illness temporarily related to the use of an (experimental) drug, whether or not it is related to the (experimental) drug. This includes:

(1) Any new clinical condition, sign, or symptom, clinically significant physical examination abnormality, or newly diagnosed event occurring during the ADR reporting period, including signs or symptoms associated with an underlying condition that was not present prior to the ADR reporting period;

(2) A preexisting condition that has worsened in severity or frequency or changed in character after the participant signed the RCT during the ADR reporting period; and

(3) Complications that occur as a result of interventions required by the protocol. An ADE may arise from any use of the investigational drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose. Any side effects, harm, toxicity, or hypersensitivity reactions that may occur in a participant in this clinical trial may also be considered AEs.

For the purposes of this protocol, events that will not be considered AEs include:

- Fluctuating signs or symptoms expected from a pre-existing medical condition (e.g., tremor in a participant with Parkinson's disease; migraine episodes) that did not worsen in severity or frequency or change in character during the ADE reporting period;
- Surgeries or medical procedures are not AEs; however, the clinical condition (new or worsened) that led to the surgery or medical procedure is the reported AE (e.g., for appendicitis resulting in an appendectomy, the appendicitis should be reported as the AE);
- Overdose without clinical signs or symptoms.

10.2 Adverse Event Reporting Period

AEs, including serious adverse events (SAEs), will be collected throughout the study period, from the time the participant signs the WIC until the EoS visit. All AEs still present at the time of study completion will be monitored by the investigator through contact with the participant until resolution or stabilization, or until the participant is lost to follow-up and can no longer be contacted. The outcome must be documented in the participant's source documents. The investigator must report

all SAEs that occur after the reporting period specified in the protocol if, in the investigator's judgment, there is a reasonable possibility that the SAE is related to the investigational product or any study procedure.

10.3 Identification of Adverse Events

If a participant reports an adverse event, it is the investigator's responsibility to obtain sufficient information to assess causality. This may require additional laboratory tests, a physical examination, telephone contact, etc.

To avoid bias in the collection of AEs, participants should be asked to answer a neutral question, such as "How are you feeling?" It is also important to ask the participant, in a non-leading manner, about changes in their health or use of concomitant medications since the last visit. This information should be collected prior to the assessments at all study visits. In addition, any symptoms/conditions reported during the assessments and considered clinically significant by the investigator will be evaluated as AEs.

10.4 Adverse Event Assessment

10.4.1 Intensity/ Severity

The medical assessment of intensity will be determined using the following definitions:

Mild: The AE is easily tolerated and does not affect usual activities.

Moderate: The AE affects daily activities, but the participant is still able to perform them.

Severe: The AE is incapacitating, and the participant cannot work or perform their usual activities.

A new event will be documented whenever the intensity of an event changes. It is important to note the distinctions between serious AEs and serious adverse events (SAEs). Severity is a classification of the intensity of a specific event (such as mild, moderate, severe); however, the event itself may be of relatively minor clinical importance (such as a severe headache).

A SAE, however, is an AE that meets any of the specified regulatory criteria required for severity designation (for example, a headache may be severe [significantly affecting the participant's usual functions], but would not be classified as severe unless it met any of the criteria for SAEs).

10.4.2 Causality and Reporting

The investigator will provide a causality assessment for all AEs using their best clinical judgment based on the available medical information regarding the event being reported. The causality assessment will be reevaluated as new information becomes available. If the investigator's causality assessment is not reported, the event will be considered "related" until such information is received. Each investigator will assess the degree to which the ADR is related to the investigational drugs using the following definitions:

Unrelated: There is no reasonable possibility that the investigational product caused or contributed to the ADE.

- The event is related to an etiology other than the investigational drug, such as an underlying disease, study-unrelated procedures, concomitant medication, or the subject's clinical condition.
- The timing of the ADE is not reasonably related to the administration of the investigational drug.

Related: There is a reasonable possibility that the investigational product caused or contributed to the AE.

- There is no compatible temporal association between the event and the administration of the investigational drug.
- Is there a biologically plausible mechanism by which the study treatment could have caused or contributed to the ADE?
- The event improves or resolves after discontinuation of the study drug without the initiation of any specific treatment for the event (withdrawal from exposure) and/or the event recurs or worsens after reintroduction of the study therapy.
- The event cannot reasonably be attributed to a concomitant or underlying disease or to other medications or procedures.

For the purposes of causality assessment, "reasonable possibility" means that, based on the investigator's medical judgment regarding the available information, there are facts or arguments suggesting a positive causal relationship.

10.4.3 Categorization of Outcomes

The outcome may be classified as: recovered/resolved (e.g., without sequelae); recovered/resolved with sequelae; not recovered/not resolved; fatal; or unknown (if follow-up is not possible).

If the outcome of an AEFI is reported as recovered/resolved with sequelae, the investigator must specify the type of sequelae on the AEFI form. If the outcome of an AEFI is reported as unknown, the investigator must specify (on the AEFI form) the rationale for selecting “unknown.”

"Fatal" must be recorded as an outcome when the AE results in death. The cause of death is required when known. If an autopsy was performed, an autopsy report must be provided. If an autopsy was not performed, a death certificate must be provided, if obtainable. Death will be reported as an outcome and not as an event. If more than one AEs is possibly related to the participant’s death, the outcome of death should be attributed to the AE that, in the investigator’s opinion, is the most plausible cause of death. All other ongoing AEs/AEGs should be recorded as unrecovered/unresolved at the time of death.

10.5 Recording and Reporting

10.5.1 Persistent or recurrent adverse events

AEs that persist continuously, without resolution, between clinical trial assessments should be recorded. A new event should be documented whenever the severity of an event changes. AEs that resolve and then recur should have each recurrence recorded separately in the medical record.

10.5.2 Diagnosis versus signs and symptoms

Whenever possible, the investigator should report a diagnosis rather than individual signs and symptoms or abnormal laboratory values. However, if a cluster of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded in the medical record. If a diagnosis is established later, all previously reported AEs based on signs and symptoms should be canceled and replaced with an AE report based on that single diagnosis, with an onset date corresponding to the onset date of the first symptom of the eventual diagnosis.

The investigator should use standard medical terminology/concepts and avoid colloquial terms and abbreviations. Only one AD term should be recorded in each event field in the medical record.

10.5.3 Pre-existing clinical conditions

A pre-existing condition is one that is present at the screening visit for this study. This condition must be recorded on the medical history form. A pre-existing condition should only be recorded as an AE if the frequency, severity, or nature worsens during the study. When recording these events in the AE clinical record, it is important to indicate the concept of change regarding the pre-existing condition, including applicable descriptors (e.g., “more frequent headaches”)

10.5.4 Clinical Laboratory Tests

Not every laboratory test with results outside the reference range qualifies as an AE. A laboratory test result should be reported as an AE if it meets any of the following criteria:

- Be accompanied by clinical symptoms
- Result in a change in the study treatment (e.g., modification of the dosage regimen, discontinuation of treatment, or discontinuation of the study)
- Result in an unplanned medical intervention
- Show a change in a parameter from a normal value to a pathological value or a further worsening of an already pathological value
- be considered clinically significant in the investigator’s opinion

It is the investigator’s responsibility to review all laboratory results. Medical and scientific judgment must be exercised to decide whether an isolated laboratory abnormality should be classified as an ADE. When evaluating such changes, the extent of the deviation from the reference range, the duration until return to the reference range—whether during continuous treatment or after discontinuation of treatment with the investigational product—and the range of variation of the respective parameter within its range must be taken into account.

The researcher is responsible for determining the clinical significance of each abnormality.

If pathological laboratory values are present at the end of the treatment phase that were not present at baseline, additional clinical or laboratory investigations must be conducted until the values return to the reference range or a plausible explanation (e.g., concomitant disease) for the pathological laboratory values is found. The investigator must decide, based on the above criteria and the participant’s clinical condition, whether a change in a laboratory parameter is clinically

significant and therefore constitutes an ADE. If the investigator considers this ADE to be serious, it must be reported as a SAE.

If a laboratory abnormality meeting the above criteria is a sign of a disease or syndrome, only the diagnosis should be recorded in the medical record. If a laboratory abnormality that meets the above criteria is not a sign of a disease or syndrome, the abnormality itself should be recorded in the medical record, along with a descriptor indicating whether the test result is above or below the normal range (e.g., “elevated potassium” rather than “abnormal potassium”). If the laboratory abnormality can be characterized by a precise clinical term according to standard definitions, the clinical term should be recorded as the AE, for example, hypercalcemia or hypoglycemia. The initial severity of the event should be recorded, and the severity should be updated at any time if the event worsens.

All pathological laboratory values/findings diagnosed during the treatment period must be reviewed by the investigator to provide a final clinical assessment in light of the dynamics of the laboratory changes/abnormalities.

10.5.5 Abnormal vital signs and other abnormalities

Laboratory results, ECGs, vital signs, and other non-standard safety assessments will be considered AEs if they meet at least one of the following criteria:

- They are associated with symptoms or result in a diagnosis (in which case, the symptom or diagnosis will be recorded as an ADR)
- Lead to discontinuation of the investigational product
- Require treatment or referral of the participant for additional off-protocol testing (retesting or titration are within the protocol procedures).

It is the investigator’s responsibility to review all vital signs, ECGs, and other safety findings. Medical and scientific judgment should be exercised to decide whether an isolated laboratory abnormality should be classified as an AE. If a clinically significant abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (e.g., hypertension) should be recorded in the medical record.

Observations regarding the same clinically significant laboratory abnormality between visits should not be recorded repeatedly, unless there are changes in the etiology. The initial severity of the event should be recorded, and the severity should be updated at any time if the event worsens.

10.6 Adverse drug reactions and reference safety information

10.6.1 Adverse drug reaction

An adverse drug reaction (ADR) is an undesirable and unintended response to a drug related to any administered dose. This definition implies a reasonable possibility of a causal relationship between the event and the investigational drug. This means that there are facts (evidence) or arguments suggesting a causal relationship.

Given that the investigational drugs have been commercially approved by ANVISA for decades, in this study only adverse reactions not yet described in ANVISA's drug dossier and assessed by the investigator as having a reasonable causal relationship with a drug (under investigation) will be considered ADRs. Thus, no reports of ADRs related to the drugs used in this study are expected.

10.6.2 Safety Reference Information

Basic safety information (RSI) forms the basis for assessing the predictability of an AEF for accelerated reports and annual safety reports, as well as for the safety monitoring of clinical trial participants by regulatory agencies (or ethics committees). In the context of this study, no ADR reports are expected, as potential adverse reactions are expected to already be described in the SIR for the investigational drugs (, and ANVISA Drug Dossier, and the package insert for the drug registered with ANVISA), except in exceptional cases, for the investigational medical products in this study.

10.7 Serious adverse event

10.7.1 Definition of a serious adverse event ( )

A SAE is defined as any adverse medical event that, at any dose:

Results in death

Be life-threatening (the term “life-threatening” in the definition of severity refers to an event during which the participant was at risk of death; it does not refer to an event that hypothetically could have caused death if it had been more severe)

Require hospital admission or prolongation of an existing hospitalization. Hospitalizations for elective surgery (i.e., a planned, non-emergency medical procedure), social hospitalizations, and hospitalizations lasting less than 24 hours are not considered SAEs

Result in persistent or significant disability/impairment

Be a congenital anomaly/birth defect

Be a significant medical event (i.e., clinically significant)

Medical and scientific judgment must be exercised to decide whether prompt reporting is appropriate in other situations, such as for medically significant events that may not be an immediate life-threatening event or result in death or hospitalization, but that may put the participant at risk or require intervention to prevent one of the other outcomes listed in the definition above. These events should also be considered serious.

Any worsening of a pre-existing condition or any new condition that meets the above SAE criteria should be considered an SAE, and the investigator is encouraged to discuss with the study coordination any SAE for which the severity assessment is uncertain or questionable.

The following situations are not considered SAEs:

Elective or pre-planned surgery for a pre-existing condition that has not worsened

Routine health evaluations requiring hospitalization not associated with a deteriorating clinical condition

Social hospitalization (homelessness, family circumstances, etc.)

Adverse reactions associated with the investigational drugs, which are expected for such drugs according to the drug dossier registered with ANVISA

Results under investigation

10.7.2 Reporting of serious adverse events

The SAE reporting period begins when the consent form is signed by the participant. The SAE reporting period ends at the 90-day visit.

The occurrence of an SAE must be reported immediately to the study monitoring committee within 24 hours of its notification via fax, email, or telephone. This includes all SAEs (regardless of their relationship to the study treatment).

A death occurring during the study or reported to the investigator, whether considered treatment-related or not, must be reported to the study monitoring committee.

Any SAE considered to have a causal relationship (e.g., related) with the investigational product and discovered by the investigator at any time after the study must be reported. The investigator must provide a rationale for the assessment of a causal relationship. All safety information obtained after the clinical database has been closed must be documented in the safety database, and the implications for handling the data in the clinical database must be evaluated on a case-by-case basis.

The onset date of an SAE is defined as the date on which the signs/symptoms/diagnoses became serious (i.e., meet at least one of the severity criteria). If a participant experiences an AE and it progresses to an SAE, a new SAE must be recorded. The resolution date of the original AE must be the same as the onset date of the SAE. However, when the AE is resolved and the pre-existing AE is still ongoing, this should be recorded as a new AE. The resolution date of an AE is defined as the time when symptoms disappear or when the event is considered chronic (e.g., sequelae) or stable and/or if the severity criteria are no longer applicable.

The investigator must complete the EAS report form and verify the accuracy of the information recorded on the EAS pages against the source documents. The sponsor's EAS report form must be completed in capital letters, using medical terminology, in English, and within the specified time limits. Any supporting documentation (e.g., hospital discharge summary, autopsy report/death certificate, etc.) must be submitted/transmitted with the SAE report form (attachment). The supporting information provided must not reveal the participant's identity beyond the agreed-upon study identifier. The investigator must ensure that the reported information is accurate and consistent.

At a minimum, the following information must be provided at the time of creating the initial SAE report:

- Study name and/or number
- The participant's number, age, and sex/gender
- The description/exact wording of the event (including the onset date of the SAE, its outcome, and the reason it was considered serious)
- Relationship to the investigational medical product (e.g., causality)

- Dose of the investigational medical product (number of packages) and dates of administration
- Action taken regarding the investigational medical product
- Severity of the event
- Name and address of the investigator
- Name of the reporter (including center name or number and country) and,
- Dated signature of the investigator or sub/co-investigator

When using electronic methods for reporting SAEs, some of the information listed above may be generated by the electronic system. Since SAEs are also AEs, the information in the AE clinical record and the SAE form must be consistent.

Follow-up information must be handled in the same manner and reported within the same timeframe as the initial SAE report. A safety contact sheet will be provided to the Investigator (before the first participant provides informed consent), detailing all applicable contact information for safety reporting. This contact sheet will be kept up to date, and any changes will be provided immediately to the Investigator.

Whenever possible, the investigator should report a diagnosis rather than individual signs and symptoms or abnormal laboratory values.

In the event of a fatal outcome, the investigator should provide a working diagnosis (the event that caused the outcome, e.g., death due to fatal myocardial infarction) rather than reporting only the death; and an autopsy report should be provided when possible. If the cause of death becomes known later (e.g., after autopsy), this working diagnosis should be replaced by the established cause of death.

All reported SAEs, regardless of their relationship to the investigational product, must be followed up until resolution or stabilization, or until the participant is lost to follow-up and can no longer be contacted. At the EoS visit, updates must be recorded and submitted. In circumstances where the investigator cannot contact the participant (or their relatives), the investigator must provide a written statement (recorded in the participant's source documents) to the study steering committee, confirming that the participant is no longer being followed up.

10.7.3 SUSARs

A suspected unexpected serious adverse reaction (SUSAR) is defined as any serious and unexpected adverse drug reaction (ADR).

For the purposes of this protocol, the occurrence of SUSARs is not expected, since the drugs were approved several years ago by ANVISA and have been used in hundreds of thousands of participants, and potential adverse reactions and/or idiosyncrasies are already well known to regulatory authorities.

The sponsor or investigator must notify the ethics committee and regulatory authorities of all SUSARs and other types of AEs (if applicable) in accordance with local safety requirements.

10.8 Special Situations

10.8.1 Definition of special situations

The following situations are defined as special:

Medication abuse: persistent or sporadic, intentional, and excessive use of the study medication by the participant (not for therapeutic purposes)

Medication error: An unintentional error in the prescribing, dispensing, or administration of an investigational medicinal product (IMP) during the study. (A medication error is any preventable event that may cause or lead to the inappropriate use of the medication or harm to the patient while the medication is under the control of the healthcare professional or the patient).

Medication misuse: Intentional and inappropriate use of an EFP by the participant for therapeutic purposes that does not conform to the dose, route of administration, and/or indication(s) specified in the protocol (e.g., the participant deliberately took the medication twice daily instead of once daily)

Drug overdose: administration of an amount of the study drug equivalent to three times the maximum dose permitted by the protocol per administration or per day.

Drug-drug interaction involving the study drug

Unexpected therapeutic or clinical benefit from the use of the study medication

Suspected AEs associated with medication errors or unauthorized use (e.g., overdose) must be reported and documented in the medical record.

10.8.2 Recording and reporting of special situations

All special situations must be documented in the participant's source documents. If a special situation leads to a serious adverse event (SAE), the event must be reported immediately within 24 hours of notification via fax, email, or telephone.

10.8.3 Exposure during pregnancy and birth events

10.8.3.1 Definition of exposure during pregnancy and birth events

Decades of experience with metformin use suggest that this medication should not be prescribed to pregnant participants without a careful assessment of the risks and benefits of its use during this phase. Thus, pregnancy is not expected during the treatment phase, and women must use contraceptive methods to prevent pregnancy (if necessary, we will provide an effective contraceptive method for use during the medication period).

When a female participant becomes pregnant during the study and has received study treatment, the pregnancy outcome must be monitored, and the safety of the mother and fetus must be assessed. Therefore, the outcome of all such pregnancies (including normal births) must be monitored and documented, even if the participant has been withdrawn from the study or the study has been terminated.

A female participant must immediately inform the investigator if she becomes pregnant during the study. The investigator must counsel the participant and discuss the risks and benefits of continuing the study medication and advise the patient on follow-up until the child is born.

The investigator is responsible for monitoring the participant and the pregnancy outcome and for reporting this information to the sponsor. Every effort should be made to collect information on the pregnancy outcome up to 90 days after delivery (or otherwise, as appropriate).

10.8.3.2 Exposure during pregnancy and recording and reporting of birth events

Pregnancies must be reported throughout the study, including up to 4 weeks after the last dose of the study drug received. Pregnancy reporting includes exposure of the female partner of a male participant. Although pregnancy is not considered an AEFI, it must be reported within 24 hours of notification by the participant. Pregnancy complications are reported as AEs or SAEs (if applicable). All pregnancies will be followed through delivery to record any SAEs. Deaths, spontaneous or elective abortions, congenital anomalies/birth defects, and AEs/SAEs occurring in newborns must be reported as SAEs. Newborns potentially exposed to the study drug through

maternal or paternal sources who present with an AEFI before, during, or after delivery (including those who were breastfed by the participating mother) will be followed up until the event is resolved (or for a period of 1 year).

11 STUDY COMMITTEES

11.1 Data Monitoring Committee (DMC)

An independent DMC will be established, composed of scientists of good reputation and experience, with no involvement in this research protocol. The DMC will act as a research advisor to monitor the safety of participants in this study. The DMC is governed by a charter that outlines the working procedures and responsibilities of the DMC. The Steering Committee will define the working procedures and responsibilities of the DMC. The charter will be agreed upon in advance by the DMC and will adhere to good research practices.

11.2 Event Adjudication Committee

The independent Event Adjudication Committee (EAC) will evaluate all events related to the study outcomes based on pre-established criteria in a prospective and blinded manner. EAC members must not be directly involved in the research and must include at least two qualified members. The EAC will not be aware of the study's treatment assignments when evaluating events. Outcome adjudication will occur continuously throughout the blinded treatment phase of the study.

12 STATISTICAL CONSIDERATIONS

12.1 Study Design

This study is a randomized, adaptive-platform Phase III clinical trial. The study evaluates the efficacy and safety of novel and repurposed therapies (investigational products [IPs]) in participants with symptoms of chronic fatigue from long COVID-19 following resolution of acute COVID-19 infection. This study evaluates various IPs for chronic fatigue syndrome from long COVID-19 under a common platform.

12.1.1 Internal Pilot Phase

Due to the rapidly evolving knowledge regarding LONG COVID, it is necessary to assess various aspects related to the study's feasibility that need to be evaluated as soon as we begin implementation.

The objective of the internal pilot phase is to identify and resolve any unforeseen feasibility issues to enhance the overall success of the research. In particular, we will assess issues related to recruitment, informed consent, medication availability and administration, and data collection and recording. There will be no analysis of clinical results at the end of this phase—since participants will be transferred to the main study and analyzed collectively. This will involve a blinded analysis of up to 10% of the target sample size, with an initial focus on the safety and tolerability of the investigational products under evaluation.

12.1.2 The Main Clinical Study

This phase involves the full implementation of the clinical trial, with the primary clinical endpoint being a reduction in perceived fatigue on the Fatigue Severity Scale (FSS). This phase is also an adaptive phase, with three interim analyses to evaluate the effects against the placebo arm. The main adaptations include:

I. Discarding the placebo arm if there is strong evidence of benefit.
II. Discard active study arms that show statistically unfavorable results.
III. Partnering with study arms of common interest from other study platforms, with the goal of obtaining responses in less time compared to conducting the study in isolation.

IV. Add new arms with investigational medicinal products that demonstrate significant potential for benefit in preclinical and Phase II clinical evidence (safety, tolerability, and potential dose-effectiveness).

There will be three interim analyses (at 25%, 50%, and 75% of the proposed sample size of 1,500 participants, with 500 participants per intervention arm) to assess the effects of the proposed interventions compared with the placebo arm.

12.2 Randomization

Participants will be randomly assigned to one of the study arms in an equal allocation (e.g., 1:1:1). Stratified permuted block randomization will be used for this study. Randomization will be stratified by participating primary care facility with varying block sizes of participants. We will use a computer-generated interactive voice/web response system to implement randomization. For each active drug arm, there will be a placebo (or active control) arm, including for the different formulations and dosages in the study.

12.3 Sample Size Calculation I

The sample size of 500 participants per arm and its operational characteristics were confirmed using closed-form, analytical, and numerical methods. The operational characteristics for the head-to-head comparison of the treatment arm versus the placebo arm in reducing the mean fatigue score measured by the Fatigue Severity Scale (1 to 9) on day 60 are shown inTable2 . For each pairwise comparison against the placebo arm, the Type I error rate (alpha) was set at 0.025 (one-sided) using a Bayesian normal-normal model. For the planning of our study, we assumed a plausible standard deviation value of 1.7, which was estimated from a recent study that evaluated the psychometric properties of the fatigue severity scale in participants recovering from COVID-19.[1]

Table2 : Simulated operational characteristics of our study for varying sample sizes

Dropout rate	Reduction in FSS score	Sample (n = 252)	Sample (n = 500)	Sample (n = 752)	Sample (n = 1000)
0.0	0.00	2.5%	2.5%	2.5%	2.5%
	0.15	13.2%	22.6%	32.3%	41.5%
	0.30	41.8%	71.2%	87.8%	95.2%

Dropout rate	Reduction in FSS score	Sample (n = 252)	Sample (n = 500)	Sample (n = 752)	Sample (n = 1000)
	0.45	76.8%	97.1%	99.7%	100.0%
2.5	0.00	2.5%	2.5%	2.5%	2.5%
	0.15	12.9%	22.0%	31.5%	40.5%
	0.30	40.6%	69.7%	86.8%	94.7%
	0.45	75.4%	96.6%	99.7%	100.0%
5.0	0.00	2.5%	2.5%	2.5%	2.5%
	0.15	12.6%	21.6%	30.8%	39.6%
	0.30	39.5%	68.5%	85.9%	94.1%
	0.45	73.9%	96.3%	99.6%	100.0%
10.0	0.00	2.5%	2.5%	2.5%	2.5%
	0.15	12.2%	20.6%	29.4%	37.8%
	0.30	37.7%	66.1%	84.0%	92.9%
	0.45	71.5%	95.4%	99.4%	99.9%

Thus, the clinical trial will recruit 1,500 participants, divided into three groups of 500 participants each. With the proposed sample size of 500 participants in each group, the study will have adequate statistical power to detect a clinically significant treatment effect. A difference of 0.45 has been suggested as the minimum clinically important difference (MID) on the fatigue severity scale in population groups of participants with multiple sclerosis and chronic fatigue.[2] Even with a 10% dropout rate, our estimated power to detect a difference of 0.45 is above 90%.

During interim analyses, the unblinded statistician advising the DMC will make recommendations regarding sample size based on the ongoing evaluation of global data and epidemiological data from Brazil and the state of Minas Gerais. We are continuously monitoring the literature for new findings relevant to chronic fatigue and to participants with long-haul COVID-19.

12.4 Statistical analysis and reporting

The analysis and reporting of results follow the CONSORT guidelines (www.consort-statement.org). The participant selection process and flow throughout the study will be summarized

using a flow diagram. The results of the analysis of participants' demographic data and baseline outcome variables (primary and secondary) will be summarized using descriptive summary measures: expressed as mean (standard deviation) or median (minimum-maximum) for continuous variables, as appropriate, and number (percentage) for categorical variables. We will adopt an intention-to-treat principle to analyze all outcomes.

Bayesian statistics is the standard statistical framework. Statistical analyses of IPs are limited to simultaneously randomized controlled data. Sequential designs with Bayesian stopping rules are expected to be used to evaluate multiple IPs. Recruitment for an IP will be stopped before the maximum sample size using pre-specified decision rules. The maximum sample size and corresponding decision rules are determined a priori by dedicated statisticians and methodologists, who will remain blinded during the conduct of the study. The statistical analysis plan (SAP) will be formulated to ensure that the expected one-sided Type I error is less than 0.025 for each treatment. We will also use multiple imputation to handle missing data. For all models, results will be expressed as the effect reported as the mean difference for continuous outcomes, odds ratios (OR) for binary outcomes, and hazard ratios (HR) for time-to-event outcomes, with their respective 95% confidence or credibility intervals.

All analyses will be performed using the latest version of R at the time of analysis. A detailed analysis plan will be developed prior to database lock. Any relevant safety data specific to Brazil will be immediately reported to our group for appropriate regulatory action.

12.5 Analysis of primary and secondary outcomes

To analyze the primary outcome of the mean fatigue score at day 90 as measured by the Fatigue Severity Scale, we will use the Bayesian normal-normal model with non-informative prior distributions for the group means. This analysis will be adjusted for prespecified covariates. For all other continuous outcomes, we will use similar modeling approaches. For all binary outcomes, we will use logistic regression for analysis. The Cox proportional hazards model will be used for all time-to-event outcomes. All analyses of secondary outcomes will be exploratory in nature, without adjustment for alpha across multiple secondary analyses.

12.6 Sensitivity Analyses

We will perform several sensitivity analyses to assess the robustness of the results, particularly for the primary outcome. These include:

i) Protocol-based analysis limited to participants who adhered to the protocol as described;

ii) Competitive risk analysis: This analysis will treat death as a competitive risk factor for any outcome;

iii) Missing data analysis: This analysis will assess the impact of missing data on the primary outcomes.

iv) We will also conduct sensitivity analyses to account for any unforeseen issues that arise during the study process and that may affect the main conclusions.

12.7 Subgroup analyses

We will conduct several subgroup analyses to assess the consistency of effects across subgroups of participants:

i. Age - Assumption that older participants will benefit more than younger ones.
ii. Gender - Assumption that female participants will benefit more than male participants.
iii. Vaccination status - Assumption that vaccinated participants will benefit more than unvaccinated participants.
iv. Comorbidities at screening:
    - Obesity status (yes or no)
    - Cardiovascular disease (yes or no);
    - Lung disease (yes or no);

Our hypothesis is that participants with the clinical comorbidities described above will benefit more than those without these clinical conditions. All subgroup hypotheses are based on emerging data from other countries indicating the differential impact of COVID-19 by age, sex, vaccination status, and the presence of clinical comorbidities at baseline. Subgroup effects will be assessed by including an interaction term between the treatment group and the subgroup variables. These interaction effects will be exploratory in nature.

12.8 Missing Data

Due to the study design and short duration, we expect to obtain data from all participants. However, in the unlikely event of missing data, they will be addressed.

12.9 Policy on pooled analysis

It is expected that data from individual participants in similar studies may be pooled in a pooled analysis. De-identified data from the present study may be made available for these purposes following discussion by the Steering Committee and in accordance with a policy of academic-scientific cooperation to find solutions for the treatment of long COVID-19.

12.11 Summary table of analyses from the website

Result	Hypothesis	Outcome Measure	Statistical analysis method
Primary Mean score measured by the fatigue severity scale on day 60 after randomization	Drug treatment will be better than placebo	Fatigue Severity Scale	Bayesian Normal-Normal analysis
Secondary Mean score measured by the fatigue severity scale on day 30 after randomization	Drug treatment will be better than placebo	Fatigue Severity Scale	Bayesian Normal-Normal Analysis
Mean score measured by the fatigue severity scale on day 90 after randomization	Drug treatment will be better than placebo	Fatigue Severity Scale	Bayesian Normal-Normal Analysis
Health-related quality of life on day 60 after randomization	Drug treatment will be better than placebo		Bayesian Normal-Normal Analysis
All-cause mortality by day 60 after randomization	Treatment will prevent overall mortality	Measuring deaths in the groups	Logistic regression
Time to all-cause mortality up to day 60 after randomization	The treatment will prevent overall mortality	Interval in days between randomization and mortality	Cox regression
Unexpected hospitalization from any cause by day 60 after randomization	The treatment will prevent hospitalization	Measurement of deaths in the groups	Logistic regression
Unexpected hospitalization for any cause up to day 60 after randomization	The treatment will prevent hospitalization	Interval in days between randomization and mortality	Cox regression
Safety of the IP	The drugs are safe in participants with long COVID	Measurement of adverse events in treatment groups	Descriptive analysis
3) **Subgroup analysis:** i) Age (young vs. elderly) (ii) Sex (male vs. female) female)	Older adults are at higher risk of complications Men are at higher risk	Risk assessment	Regression methods with appropriate interaction terms.

iv) Vaccination status v) Obesity vi) Diabetes vii) Hypertension viii) Cardiovascular disease ix) Chronic lung disease	Unvaccinated individuals are at higher risk Obesity carries a higher risk Diabetes carries a higher risk People with high blood pressure are at higher risk Lung disease carries a higher risk		
4) **<u>Sensitivity analysis</u>**	The results remain robust	Primary and key secondary outcomes (fatigue score at day 30 and day 60)	
IMPORTANT NOTES: • In all analyses, results will be expressed as estimated effects with corresponding 95% uncertainty estimates			

13 ETHICAL CONSIDERATIONS OF THE STUDY

13.1 Ethical conduct of the study

The study will be conducted in accordance with the principles of the World Medical Association's Declaration of Helsinki [229] and the International Council for Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), as amended.

The investigator must ensure the anonymity of all study participants. Each participant must be assigned a unique participant number, which must be used on all forms associated with the participant's documents or samples that will be provided to the sponsor or any party performing tests on behalf of the sponsor (e.g., blood for central laboratory testing).

All anonymous data remain the property of the Steering Committee.

13.2 Informed consent

The participants' individual medical information obtained as a result of this study is considered confidential, and its disclosure to unauthorized persons is prohibited. Participant confidentiality will be ensured by using unique participant numbers instead of names. If the results of this study are reported in medical journals or at meetings, or if they are submitted to the appropriate regulatory authorities in connection with regulatory procedures, such as applications for marketing authorization of pharmaceutical products, the participant's identity will not be disclosed.

With the participant's permission, medical information may be provided to the participant's personal physician or other appropriate medical staff responsible for the participant's well-being.

In accordance with GCP guidelines, all participants will be informed about the purpose of the research, the possible risks, and their right to withdraw from the study at any time, without any harm and without risk to their future medical care at the center. Each participant must agree to cooperate in all aspects of the study and must provide written confirmation (signed informed consent form) to the investigator prior to participation in the study. If the informed consent form is modified during the course of the study, active participants must sign the new version to continue participating in the study. For any updated or revised ICF, if applicable, the participant record must state that written informed consent was obtained for the use of the updated/revised consent form for continued participation in the clinical study. The ICF must be revised whenever there are changes to the

procedures in the protocol amendment associated with the ICF procedures or when new information becomes available that may affect the participant's willingness to participate. Each participant will receive a copy of each version of the form that they sign before and during the study.

Informed consent may be obtained in electronic format, with a copy made available to the participant. In this situation, the Ethics Committee must approve the step-by-step procedure to be adopted in the process of obtaining informed consent, which must be approved in advance so that this method may be adopted by participating centers.

No participant should participate in study activities until informed consent has been obtained. Documentation of the informed consent process and the discussion of the information provided to the participant must be included in the participant's medical record and include a statement that informed consent was obtained prior to participation in the study. Signed forms (ICFs) must remain in the participant's files and must be available for review by monitors, auditors, and/or inspectors from regulatory agencies at any time.

13.3 Research Ethics Committee (CEP)

All researchers participating in this study must be governed by an appropriate REC. The REC/CONEP system must review and approve this protocol, the TCLE, the study documents, and any information to be provided to the participant before a site may begin conducting any study-related activities.

Subsequently, the investigator is responsible for obtaining renewed approval from the CEP annually, or more frequently, in accordance with regulatory requirements and the policies and procedures established by the CEP. Copies of the investigator's annual report and other reports required to be submitted to the CEP, as well as copies of the CEP's ongoing approval, must be provided to the Steering Committee. The investigator must also inform the REC of any protocol changes or amendments, expedited SAE reports submitted to regulatory authorities, and other significant safety concerns, in accordance with the IRB policy. Written documentation of the IRB's approval of protocol amendments must be received prior to their implementation. Upon completion or termination of the study, investigators must notify their IRBs. The investigator shall comply with IRB policies throughout the study period.

14 QUALITY CONTROL AND QUALITY ASSURANCE

The integrity and quality of participant data will be ensured through training and instruction on the completion of clinical records, quality control checks, ongoing analysis of clinical data (including medical history and safety reviews), and verification and reconciliation of source data.

The investigator will also allow the Steering Committee or its audit representative, the CEP, ANVISA, or other regulatory authority inspectors to review and inspect the facilities, procedures, and all records relevant to this study. These records include, among others, the informed consent form signed by the participant, source documentation, regulatory and essential documents, clinical records, and drug accounting records.

The following measures will be taken to ensure that the study is conducted by the research center in accordance with the study protocol, GCP, and other applicable regulatory requirements:

- Meeting with the investigator
- Site Initiation Visit
- Routine monitoring of the study and the research center, if applicable
- Training on the protocol and documented GCP
- Review of medical records and questionnaires against source documents
- Collection of normal reference ranges from the local laboratory

14.1 Quality management: critical processes and data

The following processes and data were identified during the risk management activities for this study as essential to ensuring the protection of human participants and the reliability of the study results.

Throughout the study, the clinical study team will work to ensure that the clinical study is operationally feasible, focusing on the study and on activities essential to the protection of human participants and the reliability of the study results, including, but not limited to, the following:

Design and implementation of the study protocol.

Tools and procedures for collecting and processing supporting data.

Tools and procedures to ensure the rights and protection of research participants.

Activities essential for study decision-making and adherence.

15 REPORTING AND DATA RECORDING

Source documents are original documents, data, and records (e.g., case histories, physician progress notes, nursing notes, medical records, hospital records, clinical and office charts, laboratory notes, evaluation memos or checklists, pharmacy dispensing records, automated instrument data records, certified copies or transcripts after verification as accurate and complete, records maintained in the pharmacy or laboratories, and participant records). Source data are contained in the source documents and must be sufficient to reconstruct all data transcribed into clinical records and to evaluate the study. Examples of source data include clinical findings, observations, summaries of inclusion information and RCT procedures, assessment of the clinical significance of laboratory results, severity and intensity of AEs, and the investigator's judgment regarding the relationship of AEs to study medications.

The investigator must prepare and maintain adequate and accurate case records that document all observations and other data relevant to the investigation for all participants.

Source documentation must be available during the monitoring visit to verify the data entered in the eCRFs, as necessary. Source documentation must also be available for verification by auditors and/or inspectors, as necessary.

15.1 Documentation source

The investigator must maintain adequate and accurate source documents on which the case reports for each participant are based. They must be separate and distinguishable. These records must include detailed notes on:

- The medical history prior to study participation;
- Basic identifying information, such as demographic data, that links the participant's source documents;
- The results of all diagnostic tests performed, diagnoses made, therapy provided, and any other data regarding the participant's medical condition;
- The participant's exposure to the study treatment;
- All AEs and pregnancies;
- All special circumstances;
- The participant's exposure to any concomitant therapy;

- All relevant observations and data regarding the participant's clinical condition during the study;
- Verbal and written communication with the participant regarding the study treatment (including the risks and benefits of the study); the date of informed consent must be recorded in the source documentation.

All study data must be available in the source documentation.

15.2 Medical records

A clinical record is designed to record all information required by the protocol to be reported on each clinical trial participant. The investigator is responsible for ensuring the accuracy, completeness, legibility, clarity, and timeliness of the data reported in participants' clinical records. Reported data transcribed from source documents must be consistent with the source documents, or discrepancies must be explained. An explanation must be provided for all missing data.

All clinical record data and visit reports must be entered only by the clinical study staff designated by the investigator. The site staff will receive appropriate training before accessing the electronic data capture (EDC) system.

Any change or correction to a medical record will be tracked via an audit trail within the EDC system. The audit trail will contain the original data value, the new data value, the date it was changed, the user who made the change, and the reason(s) for the change.

Health records must be completed in a timely manner for the respective visit (for example, the site should not wait for a monitoring visit before entering the data). Data from clinical records and visits will be tracked and entered into a clinical database. The database system will be a secure, password-protected system with a full audit trail utility.

Participant data will be reviewed through scheduled quality checks and manually by reviewing data lists. Data that appear inconsistent, incomplete, or inaccurate will be flagged by the site for clarification. Data corrections will be updated in the database and tracked in the audit trail. AEs and concomitant medications will be coded using standardized healthcare dictionaries (e.g., MedDRA and the World Health Organization Drug Dictionary).

The investigator is responsible for reviewing, verifying, and approving all participant data (e.g., medical records and completed questionnaires).

15.3 Record Retention

The investigator must maintain adequate records for the study, including complete clinical records, medical records, laboratory reports, signed ICFs, drug distribution records, adverse event reports, information on participants who discontinued the study, all correspondence with the IRB and the research steering committee, and other relevant data.

The investigator must maintain all records at the healthcare institution. The investigator must notify in writing the transfer of any study records outside the research institution after the study's completion. The retention period for research records is that recommended by regulatory authorities

16 PROCEDURES FOR PROTOCOL AMENDMENTS OR EARLY TERMINATION OF THE STUDY

16.1 Protocol Deviation

The investigator must not perform unplanned procedures or deviate from the protocol without prior written approval, except in medical emergencies. In the event of a medical emergency, the investigator must notify the medical monitor as soon as possible. Any other changes to the protocol must be implemented as an amendment to the protocol. The criteria for describing protocol deviations and how they will be handled will be documented in the study manual.

16.2 Protocol Amendments

Changes to the protocol, except when necessary to eliminate an immediate risk to participants, shall be made only with the prior approval of the Steering Committee. Each applicable regulatory authority/IRB must review and approve the changes prior to their implementation. Approval from the regulatory authority/IRB does not need to be obtained prior to the removal of an immediate risk to participants.

16.3 Study termination

The Steering Committee reserves the right to terminate the study in its entirety or at a single site at any time. Reasons for termination may include (but are not limited to) unsatisfactory enrollment of participants in terms of quality and/or quantity, the site's failure to meet the requirements of the protocol or GCP, or inaccurate and/or incomplete data recording.

In the event of study termination, the Steering Committee and the investigator must ensure that due consideration is given to protecting the interests of the subjects. Both parties must organize the procedures individually following the review and site visit, and in accordance with the study contract.

Based on its analysis of the data, the DMC may make recommendations to discontinue the study, as indicated in the DMC's internal bylaws. The Steering Committee will determine whether the study should be discontinued early.

The study may be terminated or suspended at the request of regulatory authorities.

17 PUBLICATION AND DATA SHARING POLICY

Data generated through this research protocol belong to the Steering Committee. No data may be disclosed or published without the prior consent of the Steering Committee. The confidentiality agreement to be established with the participating research centers will set forth the publication policy.

In accordance with applicable laws and regulations, the sponsor will record and publicly provide all mandatory information related to this study, including, to the extent and within the timeframes required, a summary of the clinical trial data and results.

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