STUDY PROTOCOL

An Open Label Study in Adults to Test the Efficacy of Mitoquinone/Mitoquinol Mesylate to Prevent Severe Viral Illness

NCT05381454

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Principal Investigator:

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CLINICAL PLAN

Summary

We conducted a clinical study that assessed safety and efficacy of mito-MES as post-exposure prophylaxis against viral infections including RSV and SARS-CoV-2 in adults. Given extensive published data regarding safety of mito-MES in humans (presented herein) and urgent need to develop novel oral antiviral agents, we believe that an open label exploratory safety and efficacy study will obtain proof of concept data whether mito-MES has antiviral activity in humans. This is a feasibility study to determine the safety and efficacy of the treatment with mito-MES 20 mg daily for up to 14 days to prevent development of severe clinical symptoms of any viral infection in high-risk close household contacts of cases with viral infection. Primary efficacy measures will be development of viral illness (YES/NO) throughout the study period. Secondary endpoints will be new onset fever (T> 100.3 F or 38C), duration of ANY of at least three respiratory/systemic symptom of viral illness in days and severity of viral illness based on a quantitative score system within 14 days after exposure.

The team will post advertisements on social media seeking both individuals who have been newly diagnosed, as well as people who have recently been exposed to a suspected case of viral infection. Participants within the US who meet the inclusion criteria can enroll. The study team will also actively seek out such new cases by building referral pathways from clinics, schools, communities, Emergency Departments, inpatient units, occupational health departments, and public health authorities in the vicinity of the study site. Once the list of

exposed contacts for each case is identified, the study team will make multiple attempts to contact them for up to 24 h, in order to obtain their decision about trial participation. Enrollment of exposed persons will typically occur by first identifying suspected respiratory viral infection, and then conducting contact tracing to define a ring of exposed contacts around those index cases.

Title of Study.

An Open Label Feasibility study in adults to test the safety and efficacy of mitoquinone/mitoquinol mesylate as prophylaxis for development of severe viral illness

Investigators/Study Center:

Name and address and statement of the qualifications of investigator

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For qualifications see attached curriculum vitae

Name and address of the research facilities to be used

Theodoros Kelesidis, MD, PhD, Msc University of California, Los Angeles

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Name and address of reviewing Institutional Review Board

UCLA OHRP https://ohrpp.research.ucla.edu/

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3.4. Phase of Development

Phase I/II efficacy and safety trial

3.5. Objectives:

We propose the use of Mito-MES as drug (FDA IND status) as a novel therapy for viral infections including RSV and SARS-CoV-2. Our proposed use of Mito-MES can be readily advanced to the clinic. In this project, we will focus on the design of an open label phase I/II efficacy study to test the safety and efficacy of Mito-MES to prevent the development and progression of viral infections including RSV and SARS-CoV-2 after high-risk exposure to a person with suspected viral infection (post exposure prophylaxis study). We focus on post exposure prophylaxis study in adults (see study design) and not acute treatment study, since a post exposure prophylaxis study design may require a much smaller sample size to show efficacy and may be more feasible with current resources compared to an acute treatment study.

Primary objectives:

- To determine the efficacy of the treatment with mito-MES 20 mg daily for up to 14 days to prevent development of any viral infection (Yes/no) in high-risk close household contacts of cases with viral infection
- To determine the efficacy of the treatment with mito-MES 20 mg daily for up to 14 days to prevent development of severe clinical symptoms of any viral infection in high-risk close household contacts of cases with viral infection.

Viral infection will be defined clinically based on history obtained by the investigator who is an infectious diseases physician and stringent criteria will be followed. Viral infection will be defined as presence of at least **two new onset** independent symptoms or signs (fever) of respiratory disease that cannot be attributed to bacterial cause. For example, the combination of coryza (runny nose) and sore throat is very characteristic of a viral illness since there is no bacterial "rhinitis". The combination of fever and cough is not specific enough presentation since it can be attributed also to bacterial bronchitis or pneumonia. A study participant with chronic allergies or chronic sinusitis or asthma will not be included in the study since they may have chronic runny nose or headache or cough.

Primary measures of efficacy (will be assessed in ALL the study participants):

The following **primary** outcomes are based on criteria that can be obtained reliably and consistently for all the study participants (based on history and thermometer).

- Development of any symptom of viral infection within 14 days after exposure (YES/No).

- **Severity of viral illness based on a quantitative score system**. Each of the above 14 symptoms will be given a score based on severity: 1 for mild, 2 for moderate, 3 for severe. Then a total severity score will be estimated (range of score is 0-42). Common example: a person with mild coryza, sore throat and cough will be given a score of 3.

Secondary measures of efficacy (will be assessed in study participants but some endpoints such as diagnostic tests may not be available in all the study participants):

The following secondary outcomes are based on **either non quantitative** criteria (categorical) or criteria that may not be available for all the study participants (for example FDA approved diagnostic tests). The study will not pay for any diagnostic tests. These diagnostic tests for viruses may be available in the setting of real world setting and clinical care of each participant (for example if performed by the participant or ordered by a doctor in the setting of clinical care).

- Development of **new onset fever** (T> 100.3 F or 38C) based on documented measurements of temperature on a daily basis throughout the duration of the study. Temperature will be recorded in a diary and recording temperature would be a requirement for study participation.
- **Duration** of ANY of at least three respiratory/systemic symptom of viral illness in **days** (Symptom 1: fever, Symptom 2: cough, Symptom 3: coryza, Symptom 4: sore throat, Symptom 5: shortness of breath, Symptom 6: chills, Symptom 7: fatigue, Symptom 8: loss of smell or taste, Symptom 9: myalgias, Symptom 10: arthralgias, Symptom 11: headache, Symptom 12: nausea, Symptom 13: vomiting, Symptom 14: diarrhea

- Confirmation of viral infection based on a FDA approved diagnostic test (for example respiratory panel PCR that also detects RSV and influenza or other respiratory viruses, rapid antigen test for SARS-CoV-2, PCR for SARS-CoV-2).
- Symptomatic viral infection: proportion of participants with fever, cough, or other respiratory/systemic symptoms (including but not limited to fatigue, loss of smell or taste, myalgias, arthralgias, shortness of breath, sore throat, headache, chills, coryza, nausea, vomiting, diarrhea).
- Symptomatic moderate to severe viral infection. Based on established score system a viral illness will be categorized as moderate or severe if the participant grades at least 2 different symptoms as “moderate” or “severe”.
- Symptomatic PCR confirmed SARS-CoV-2 infection
- Need for oxygen therapy (days)
- Hospitalization: Days of hospitalization attributable to viral disease: The median number of days (or partial days) spent admitted to an acute care hospital
- Respiratory failure requiring ventilatory support attributable to viral disease: The median number of days (or partial days) requiring (i) non-invasive or (ii) invasive ventilation
- Mortality (proportion of participants who die) attributable to viral disease and all-cause mortality

Outcome measures:

Adverse events (day 7, 14): the proportion of participants exhibiting adverse events of any grade as defined using the established[87] grading scales for the severity of adverse event, such as the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers

Enrolled in Preventive Vaccine Clinical Trials https://www.fda.gov/media/73679/download).

Design of Study:

Overview of study design

Open label clinical trial (intervention with a diet supplement) to evaluate the safety and efficacy of treatment with mitoquinone/mitoquinol mesylate (mito-MES) (20 mg daily) daily to prevent any viral infection in high-risk close contacts of cases with possible viral infection.

Diagnosis and Key Subject Selection Criteria (Exclusion and Inclusion):

Inclusion criteria

All enrolled participants must meet the following criteria:

1. **Age 18-65 years old**. Rationale: We will recruit 18-65 years old participants to increase feasibility of the study and to ensure the study results are generalizable. Participants older than 65 years old have higher incidence of many comorbidities including kidney, heart and liver disease. It would be important to first document safety in the setting of viral infection in the absence of established kidney, liver and heart disease and other major morbidity.
2. **Asymptomatic** (no symptoms of viral infection) on study entry.
3. Exposure to a person with at least **two new onset** independent symptoms or signs (fever) of respiratory viral disease. Viral infection will be defined clinically based on history obtained by the investigator who is an infectious diseases physician and stringent criteria will be

followed. Viral infection will be defined as presence of at least **two new onset** independent symptoms or signs (fever) of respiratory disease that cannot be attributed to bacterial cause. For example, the combination of coryza (runny nose) and sore throat is very characteristic of a viral illness since there is no bacterial “rhinitis”. The combination of fever and cough is not specific enough presentation since it can be attributed also to bacterial bronchitis or pneumonia. A study participant with chronic allergies or chronic sinusitis or asthma will not be included in the study since they may have chronic runny nose or headache or cough.

4. **High risk exposure without use of masks to suspected case of viral infection** (based on history and the study definition in the household within 5 days prior to study entry. High-risk exposure was defined as prolonged (>24 hour) and intimate (< 6 feet) exposure to index case *without personal protective equipment (e.g., face mask)* in *poorly ventilated indoor* areas. To increase chances that differences in treatment efficacy between the compared groups will be shown will as small sample size as possible, the study team enrolled participants with particularly high risk exposure to the index case defined as: a) direct care for the index case; b) had close direct physical contact with the index case; c) lived with the index case; d) had close indoor contact (within 6 feet), with or without direct physical contact, for at least 24 hours; e) had direct contact with infectious body fluids, including oral secretions, respiratory secretions, or stool. For example, we included several parents that were exposed to infected children (who also infected their siblings) and included an extended window of recurrent and continuous high-risk exposure to independent confirmed SARS-CoV-2 infections within the household. In these particularly high-risk exposures, there was direct contact with children during childcare and while the children were symptomatic (fever, cough, coryza, fatigue);
5. **With at least 4 COVID-19 diagnostic tests in case of negative test results.** To minimize false negative tests, we included participants who had multiple independent SARS-CoV-2 diagnostic tests with sensitivity >90% per test;

6. Adequate renal function determined by the Cockcroft-Gault formula for creatinine clearance (>60 mL/min/1.73 m2
7. Able and willing to communicate in English
8. Able and willing to provide informed consent to take part in the study
9. Able and willing to provide medical/surgical history
10. Availability to have follow up for all study visits, barring unforeseen circumstances

In addition, for the *mito-MES group*, the following inclusion criteria were applied:

1. Initiation of Mito-MES within 5 days since exposure to index case. To ensure that a study participant met this inclusion criterion, the study participant should have had high-risk close contact with a confirmed COVID-19 case within the past 1–4 days prior to study entry. This 4-day window would also allow for up to 24-hour period for initiation of intervention (mito-MES) so that mito-MES would be started within 5 days since exposure to index case;
2. Successfully contacted by the study team within 24 h of study team notification of the relevant index COVID-19 case. This time window is necessary because the efficacy of PEP may be dependent on the timing of its initiation. Every effort was made by the study team to include study participants who would be able to initiate mito-MES as soon as possible after notification of exposure to index case of SARS-CoV-2 infection. For many study participants, Mito-MES was provided immediately after informed consent so that it would be available for *immediate* use after *anticipated* exposure to confirmed case of SARS-CoV-2. It is well established that antivirals have highest efficacy when given *as soon as possible* (ideally within 48 hours). This design with immediate initiation of Mito-MES within 72 hours post exposure is different than the study design in a randomized controlled Phase 2/3 EPIC-PEP (Evaluation of Protease Inhibition for COVID-19 in Post-Exposure Prophylaxis) of paxlovid given within *5 days* post exposure from SARS-CoV-2 that failed to demonstrate therapeutic

efficacy (unpublished data). Time of initiation of the post-exposure prophylaxis (PEP) treatment is critical in the setting of emerging SARS-CoV-2 variants that may establish infection in less than 5 days.

3. **Ineligible for FDA approved alternative treatments** to prevent progression to severe viral infection including COVID-19 based on established criteria https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html. Rationale: If the study participant has confirmed exposure to SARS-CoV-2, the participant will be allowed to enroll only if he/she is eligible for other FDA approved treatments such as monoclonal antibodies or other antivirals for reasons such as personal choice, allergies or lack of availability.

11. Willing to follow sexual abstinence during study period or highly effective method of contraception. Rationale: Since the study drug (mito-MES) has not been studied in pregnant women, women and men of child bearing potential should use a barrier method that prevents pregnancy in combination with a highly effective method of contraception. Highly effective methods are:
    - oral contraceptive
    - Use of condoms during any sexual intercourse during the study period
    - intrauterine device
    - intrauterine hormonal releasing systems
    - Bilateral tubal ligation/occlusion
    - Vasectomized partner provided that partner is the sole sexual partner of the participant and that the vasectomized partner has received medical assessment confirming surgical success.

Given how essential it is to start potential treatment as soon as possible after high-risk exposure to a viral infection, participants will be allowed to participate without waiting for the results of a

diagnostic test such as SARS-CoV-2 FDA approved test (can delay initiation of treatment for at least 24-48 hours). If a participant has a positive SARS-CoV-2 during the study, then the participant will be allowed to continue the study only if not eligible for another FDA approved treatment for COVID-19, given the potential favorable antiviral and anti-inflammatory effects of mito-MES in SARS-CoV-2 infection. However, these participants with a positive SARS-CoV-2 test will not be included in the final analysis of this PEP trial. The data obtained from the participants with a positive SARS-CoV-2 test will set the foundation for future studies of use of mito-MES as treatment for acute COVID-19.

Exclusion criteria for all study participants:

Participants who meet any of the following criteria at screening will be excluded from the study:

1. Use of Coenzyme Q10 or Vitamin E < 120 days from enrollment
2. Women with variations in physiological functions due to hormones that may effect immune function and (transgender, **pregnant**, breastfeeding)
3. Pregnant (based on history) or lactating. If the possible study participant is female and sexually active and there is a possibility based on history (timing of most recent sexual intercourse and timing of menstrual period) that the possible study participant is pregnant, then the participant will be excluded from the study. Rationale: Additional pregnancy testing will further delay the initiation of treatment and will further compromise feasibility of the study given limited resources.
4. Absence of significant clinical disease **clinical diseases** [e.g., cardiovascular disease (such as coronary artery/vascular disease, stroke), heart disease (such as congestive heart failure, cardiomyopathy, atrial fibrillation), lung disease (such as chronic obstructive pulmonary disease, asthma, bronchiectasis, pulmonary fibrosis, pleural effusions), kidney disease (glomerular filtration rate or GFR less than 60 ml/min/1.73 m2), liver disease (such as cirrhosis, hepatitis), cancer, major immunosuppression (such as history of transplantation,

HIV infection*)*] based *on history*. Rationale: **Significant clinical** diseases can confound interpretation of results for safety and efficacy (e.g., symptomatic viral infection). For example, a patient with known major lung disease such as chronic obstructive pulmonary disease (COPD) or heart disease may have chronic cough and shortness of breath at baseline and these confounders would compromise interpretation of results whether the participant will develop symptomatic viral infection or whether the treatment has lung or heart toxicity. If this pilot clinical trial will provide promising results, future larger clinical trials can include participants with significant comorbidities such as people with coronary artery disease and established lung disease or cancer. Participant screening, informed consent, and follow-up will be exclusively internet-based with appropriate regulatory and research ethics board approvals. In-person participant follow-up will not be conducted to facilitate social distancing strategies and reduce risks of exposure to study personnel. Thus, physical exam, blood and urine chemistries will not be performed to independently confirm the absence of significant clinical diseases.

5. History of known gastrointestinal disease (such as gastroparesis) that may predispose patients to nausea
6. History of auto-immune diseases
7. Chronic viral hepatitis
8. HIV infection or other chronic viral infection (e.g. HTLV).
9. Use of systemic immunomodulatory medications (e.g. steroids) within 4 weeks of enrollment
10. Any participant who has received any investigational drug within 30 days of dosing
11. History of underlying cardiac arrhythmia
12. History of severe recent cardiac or pulmonary event
13. A history of a hypersensitivity reaction to any components of the study drug or structurally similar compounds including Coenzyme Q10 and idebenone
14. Unable to swallow tablets

15. Use of any investigational products within 4 weeks of enrollment
16. Any other clinical condition or prior therapy that, in the opinion of the investigator, would make the patient unsuitable for the study or unable to comply with the study requirements. Such conditions may include, but are not limited to, current or recent history of severe, progressive, or uncontrolled renal, hepatic, hematological, gastrointestinal, endocrine, pulmonary, neurological, or cerebral disease

Treatments

Mito-MES (called “MitoQ” and provided by MitoQ Ltd) is commercially available as 5 mg capsules. Thus, a dose of 5 mg would require 1 capsule and a dose of 20 mg would require 4 capsules.

Intervention in adults: Exposure of a potential study participant to a viral infection (whether it was **confirmed** by an FDA approved diagnostic test or not) in a close contact within the household of the study participant and which may cause severe disease in humans (such as SARS-CoV-2 infection or influenza). Intervention would be 20 mg (4 drug capsules) PO daily initiated within 5 days post exposure and taken daily for up to 14 days.

Dose Justification

Dose justification

In a randomized controlled clinical trial of patients with Hepatitis C (ClinicalTrials.gov Identifier: NCT00433108) mito-MES 40 mg orally daily had anti-inflammatory activity (reduced hepatitis) in

an epithelial tissue (liver). Mito-MES is commercially available as a dietary supplement in dose of 10 mg orally daily. The dose of mito-MES that is typically used in clinical trials in humans is 20 mg orally daily. It is likely that a higher dose (40 mg daily) is more effective against SARS-CoV-2 infection (better antiviral and anti-inflammatory effects) than the lower dose (20 mg daily). Thus, in a proof-of-concept initial study we will test the tolerability and efficacy of the 20 mg dose of mito-MES that can be well tolerated in humans over a period of 14 days (20 mg PO daily). The 80 mg oral dose of mito-MES daily is also well tolerated based on clinical trials in humans but gave higher incidence of gastrointestinal side effects.

Justification of 20 mg dose for penetration in respiratory mucosa.

Oral MitoQ can achieve adequate levels in the respiratory mucosa that can inhibit establishment of early SARS-CoV-2 infection

One important consideration is whether oral MitoQ can achieve adequate tissue levels in the upper respiratory mucosa to inhibit establishment of SARS-CoV-2 infection. MitoQ is the only mitochondria-targeted antioxidant compound currently approved for human use that can safely be delivered long-term to humans as oral formulation[23] and has been used in clinical trials for oxidative damage-related states such as liver disease[24], Parkinson disease, vascular and physiological function in older adults (www.clinicaltrials.gov)[25,26]. MitoQ is a reasonably bioavailable form of the naturally-occurring antioxidant ubiquinone/coenzyme Q_{10} conjugated to a lipophilic cation (triphenylphosphonium [TPP])[28,29]. CoQ_{10} is one of the mitochondrial respiratory chain coenzymes, protects lipid membranes from oxidative stress[65] and is important for metabolism especially in metabolically active cells like epithelial cells[30]. In contrast to other antioxidants that have proven largely unsuccessful for prevention and/or treatment of human disease[66,88-90] because they have short half-lives[66,67] and do not penetrate well into the cells (to

reach the cellular sources of ROS at concentrations that reduce or prevent their adverse effects), the lipophilicity and positive charge of the TPP cation help drive MitoQ to the inner mitochondrial membrane, where it accumulates at levels 100-1,000-fold higher than in the cytosol of cells. This optimally positions MitoQ to reduce mitochondrial oxidative stress.

In mice, MitoQ accumulates in tissues including mitochondria-enriched epithelial tissues like liver and kidney at 50-700 pmol/g (wet weight) following ≥10 days of oral MitoQ supplementation[35]. Pharmacokinetic analysis of orally administered alkyltriphenylphosphonium cations in mice determined that a single oral dose produces levels of ~2.5-7.5 nmol/g wet epithelial tissue (kidney or liver)[35]. Importantly the upper airway respiratory epithelium, the point of entry of SARS-CoV-2, is enriched in mitochondria[79] like other epithelium tissues where MitoQ levels have been determined (like kidney and liver). Although levels of MitoQ in respiratory epithelium per se have not been determined *in vivo*, MitoQ has been shown to specifically lead to reduced inflammation and proliferation in airway smooth muscle cells of mice and humans[80]. MitoQ also has good tissue penetration and therapeutic efficacy in other epithelial tissues in mice including lung[31,32,80] and intestine[81]. Following oral administration, the absorption of Mitoquinone-C10 from the rat GI tract was fast. The peak plasma concentration of Mitoquinone-C10 occurred within 1 h of oral a administration and then declined slowly over time with an elimination half-life based on post 4 h data of about 14 h[69]. In a rat tissue distribution study, mitoquinone was rapidly distributed to tissues, with a brain: plasma ratio of 1:10 after 10 minutes [78]. Given that in rodents the MitoQ tissue levels in brain are at least 3 times lower than epithelial tissues (kidney and liver) [35], the plasma levels of MitoQ *in vivo* may partially reflect levels in epithelial tissues.

These studies have established a pharmacokinetic model of MitoQ. After absorption from the gut into the bloodstream, orally administered triphenylphosphonium cations are taken up into all tissues through the lipid bilayer of the plasma membrane, assisted by the plasma

membrane potential. From the cytosol most lipophilic cations are taken up into mitochondria, driven by the large membrane potential. After several days of oral intake, the cation concentration within mitochondria comes to a steady-state distribution with circulating blood levels. At this point the mitochondrial concentration will be several hundredfold higher than that in the bloodstream, and the rate of oral absorption of the compounds will match the rates of excretion into the urine and bile. The mitochondrial pool of compound is in dynamic equilibrium and once oral uptake stops the accumulated cations will reequilibrate back into the bloodstream and be relatively rapidly excreted[35]. Prolonged administration of MitoQ leads to higher tissue concentrations that may reflect retention of mitoquinone in the mitochondrial phospholipid membranes.

In humans, a single 80 mg oral dose of MitoQ results in a maximal plasma concentration of 33 ng/mL ~1 hour after administration[29], similar to therapeutic levels of statins[82,83]. A single 40 mg oral dose of MitoQ resulted in plasma concentration of 2 ng/ml after 24 hours[25,26]. Given that levels of MitoQ in epithelial tissues is at least 3 times higher than plasma levels (based on animal data), a concentration of MitoQ at the nM level can be achieved with a single dose of MitoQ. Chronic supplementation of MitoQ at 40 or 80 mg/day results in an average elevation of fasting plasma MitoQ concentrations of ~5 ng/mL after ~10 days with no significant difference between doses[25]. The levels of plasma mitoquinone are variable, probably because of differences in absorption from the gut, effect of subsequent food consumption and rapid clearance of the molecule from the plasma to tissues[35]. Pharmacokinetics of the much less bioavailable CoQ10 have not been studied at the tissue level[84]. However, oral administration of CoQ10 in eight patients with COPD at 90 mg/day for 8 weeks improved oxygenation[85], suggesting adequate penetration in the lung tissue. Doses as high as 230 mg/kg/day are non-mutagenic and non-toxic in mice[29,71]. Using the FDA recommended surface area conversion factor[72,73], this is equivalent to a dose of ~1,119 mg/day for an average 60 kg human. This dose

is several magnitudes (~60-fold) higher than the dose use in the current study (20 mg/day). Phase II clinical trials with MitoQ have been completed in humans and have demonstrated that MitoQ can be administered to humans for up to a year at doses of 40 and 80 mg/day without any serious adverse events. Importantly, a low incidence of subject attrition due to GI discomfort (<5%) was observed with a 40 mg/day dose across two clinical trials[25,26]. A dose of 20 mg MitoQ once a day with breakfast after an overnight fast was used for post-exposure prophylaxis against SARS-Co-V-2 to maximize absorption and minimize any potential gastrointestinal symptoms[25,29]. This dose was also used in a recent clinical trial with MitoQ vs placebo supplementation in healthy, late middle aged older adults[27].

In summary, the above published evidence suggests that a single oral dose of MitoQ (20 mg daily) can achieve adequate levels (in the range of at least nM) in the upper respiratory mucosa. Our *in vitro* data suggest that MitoQ has potent antiviral activity in human lung epithelial cells at the nM range. Thus, it is biologically plausible that MitoQ can inhibit establishment of early SARS-CoV-2 infection in the upper airways and MitoQ can be a potentially useful postexposure prophylaxis strategy against SARS-CoV-2 infection. Postexposure prophylaxis against SARS-CoV-2 will be more efficacious when taken early (< 5 days post exposure)[86].

Duration of treatment justification

The duration of treatment for postexposure prophylaxis will be 14 days. We will use the maximum possible duration of treatment for postexposure prophylaxis so that if we find lack of efficacy in this clinical trial this can be attributed to lack of therapeutic efficacy of the antiviral and not due to suboptimal duration of treatment.

Main Parameters of Safety

Adverse events (day 7, 14): the proportion of participants exhibiting adverse events of any grade as defined using the established[87] grading scales for the severity of adverse event, such as the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials https://www.fda.gov/media/73679/download).

Main Parameters of Efficacy

Primary measures of efficacy (will be assessed in ALL the study participants):

The following **primary** outcomes are based on criteria that can be obtained reliably and consistently for all the study participants (based on history and thermometer).

- Development of any symptom of viral infection within 14 days after exposure (YES/No).
- **Severity of viral illness based on a quantitative score system**. Each of the above 14 symptoms will be given a score based on severity: 1 for mild, 2 for moderate, 3 for severe. Then a total severity score will be estimated (range of score is 0-42). Common example: a person with mild coryza, sore throat and cough will be given a score of 3.

Secondary measures of efficacy (will be assessed in study participants but some endpoints such as diagnostic tests may not be available in all the study participants):

The following secondary outcomes are based on **either non quantitative** criteria (categorical) or criteria that may not be available for all the study participants (for example FDA approved diagnostic tests). The study will not pay for any diagnostic tests. These diagnostic tests for viruses may be available in the setting of real world setting and clinical care of each participant (for example if performed by the participant or ordered by a doctor in the setting of clinical care).

- Development of **new onset fever** (T> 100.3 F or 38C) based on documented measurements of temperature on a daily basis throughout the duration of the study. Temperature will be recorded in a diary and recording temperature would be a requirement for study participation.
- **Duration** of ANY of at least three respiratory/systemic symptom of viral illness in **days** (Symptom 1: fever, Symptom 2: cough, Symptom 3: coryza, Symptom 4: sore throat, Symptom 5: shortness of breath, Symptom 6: chills, Symptom 7: fatigue, Symptom 8: loss of smell or taste, Symptom 9: myalgias, Symptom 10: arthralgias, Symptom 11: headache, Symptom 12: nausea, Symptom 13: vomiting, Symptom 14: diarrhea
- Confirmation of viral infection based on a FDA approved diagnostic test (for example respiratory panel PCR that also detects RSV and influenza or other respiratory viruses, rapid antigen test for SARS-CoV-2, PCR for SARS-CoV-2).
- Symptomatic viral infection: proportion of participants with fever, cough, or other respiratory/systemic symptoms (including but not limited to fatigue, loss of smell or taste, myalgias, arthralgias, shortness of breath, sore throat, headache, chills, coryza, nausea, vomiting, diarrhea).
- Symptomatic moderate to severe viral infection. Based on established score system a viral illness will be categorized as moderate or severe if the participant grades at least 2 different symptoms as “moderate” or “severe”.

- Symptomatic PCR confirmed SARS-CoV-2 infection
- Need for oxygen therapy (days)
- Hospitalization: Days of hospitalization attributable to viral disease: The median number of days (or partial days) spent admitted to an acute care hospital
- Respiratory failure requiring ventilatory support attributable to viral disease: The median number of days (or partial days) requiring (i) non-invasive or (ii) invasive ventilation
- Mortality (proportion of participants who die) attributable to viral disease and all-cause mortality

3.12 Experimental protocol of study

Recruitment:

Participants in the US will be recruited voluntarily through UCLA IRB-approved protocols. Participants will be informed about this study and given IRB-approved flyers so that they may call on their own to be considered for this study. Involvement will be strictly voluntary, and the subjects will have the right to refuse participation at any time. Recruitment of participants will be coordinated through investigators. Subjects will undergo assessments by experienced, personnel to determine their eligibility to participate in these studies. Subjects will also be interviewed by trained staff using standardized data collection forms and questionnaires, to providing detailed demographic, clinical and medication data along with history of co-morbidities. We anticipate recruiting participants as described in the Inclusion Criteria.

The team will post advertisements on social media seeking both individuals who have been newly diagnosed, as well as people who have recently been exposed to a suspected case of viral infection. Participants within the US who meet the inclusion criteria can enroll. The study team will also actively seek out such new cases by building referral pathways from clinics, schools,

communities, Emergency Departments, inpatient units, occupational health departments, and public health authorities in the vicinity of the study site. Once the list of exposed contacts for each case is identified, the study team will make multiple attempts to contact them for up to 24 h, in order to obtain their decision about trial participation.

Enrollment of exposed persons will typically occur by first identifying suspected respiratory viral infection, and then conducting contact tracing to define a ring of exposed contacts around those index cases.

Participant screening, informed consent, and follow-up will be internet- or telephone- based with appropriate regulatory approvals. In-person participant follow-up will not be conducted to reduce risks of exposure to study personnel and to ensure feasibility. Thus, physical exam, blood and urine chemistries will not be performed to confirm eligibility.

Special classes or vulnerable populations:

No special classes of participants or vulnerable populations (children under the age of 18, pregnant women, institutionalized individuals, etc) will be included in this research. In general, individuals who meet all of the inclusion criteria and none of the exclusion criteria are eligible for enrollment into this study irrespective of sex, race, or ethnicity. Specifically, no racial or ethnic group will be excluded from participation in this study, and a broad diversity of participants is encouraged. The participants will likely reflect the demographics of the population in US and the Greater Los Angeles area. This population includes various ethnic groups, including African Americans and Hispanics. We will prioritize recruitment from underserved Californians who tend to have large households. Every effort will be made to encourage the participation of women. Women with variations in physiological functions due to hormones (transgender, breastfeeding,) will be excluded from this study. Women who are pregnant or it is possible based on history that

they will become pregnant will not be included due to unknown safety of the mitochondrial antioxidant MitoQ in pregnant women. No potential research participant will be excluded based on race/ethnicity.

The historic mix of volunteers for research studies previously conducted at the project site is generally representative of the demographics of local populations. Every attempt will be made to ensure equal access to all racial and ethnic groups to participate in this research project, and although study flyers will not target specific racial/ethnic populations, they will be distributed across all available local areas. We will recruit participants with representation across a wide range of age groups with both genders and major race/ethnicities included.

Children under 18 will not be included in this study because the inflammatory and immunologic milieu may vary greatly between children and adults for some of the parameters under investigation, clouding the interpretation of results in this research study. Participants less than 18 years of age will also be precluded from volunteering due to unknown safety of the mitochondrial antioxidant mito-MES in children. For these reasons, separate studies in children should be performed that are beyond the scope of this project.

Retention:

Site investigators are trained about the importance of retention and steps to prevent missing data. To increase subject retention, all subject questions/concerns will be addressed in detail during the consent process. The consent forms will include a statement educating patients about the continued scientific importance of their data even if they discontinue study treatment early. The protocols and informed consent forms will clearly differentiate treatment discontinuation from study withdrawal. There will be constant communication will occur between research staff and participants through phone-call and email reminders prior to all upcoming

visits. Several approaches will be implemented to retain patients who fail to actively maintain contact with the investigator (e.g., telephone calls, texts, and emails to the patient and close contacts; vital records search). The study will be conducted through the phone which is easily accessible to participants. All patients who maintain consent will be followed for additional outcome information and will remain in the study through the end of the study period for all important safety and efficacy assessments. The only reasons for study withdrawal will be withdrawal of consent and loss to follow-up. Sponsors and investigators will encourage patients who discontinue therapy to remain in the study and to continue follow-up for key outcomes. Through these strategies, the investigators have recruited participants with specific interest in participating in research, leading to high rates of retention of study subjects, with high level of retention of volunteers in clinical studies even without the need for financial incentives.

Assessment of symptom severity of viral infection (including COVID-19(

Assessment of symptom severity of viral infection including COVID-19 will be performed based on the FDA guidance on Assessing COVID-19-Related Symptoms in Outpatient Adult and Adolescent Subjects in Clinical Trials of Drugs and Biological Products for COVID-19[91]. Patient-reported outcome (PRO) instruments will be used to assess COVID-19-related symptoms[91]. PRO assessments of COVID-19-related symptoms will be done at least every 24 hours and will be conducted at the same time each day. Electronic data collection systems will be used with reminders to trial subjects to complete the PRO instrument to minimize missing data and provide time stamps of completion. Alternatively, if a paper-based diary is used, the study team will send reminders (e.g., phone calls, text messages, email) to trial subjects[91]. A set of common viral-related symptoms (see **Table 1**) will be included in the daily PRO assessments of all trial subjects regardless of which symptoms a subject had at baseline, as new symptoms may appear following the baseline assessment. Each symptom will be scored individually using the

following response options and scoring values. The score values will not be included within the response options presented to trial subjects to avoid confusing subjects. Response scales include verbal descriptors (e.g., none, mild, moderate, severe) because the absence of verbal descriptors may create difficulty in interpretation in this context of use. Accordingly, use of response scales such as visual analogue scales and 0–10 numeric rating scales will be avoided since they may result in interpretation difficulties in this context. The investigators will conduct an evaluation to ensure the PRO instrument's basic comprehensibility and usability before implementation in a trial to mitigate risk of poor instrument performance. Efficacy endpoint based on COVID-19 symptom severity will be the time to sustained clinical recovery assessed over an appropriate duration. Sustained clinical recovery will be defined as occurring when no key COVID-19-related symptom scored higher than a prespecified threshold over a clinically meaningful time period (as documented using a PRO instrument). To accurately evaluate clinical benefit, we will include as trial entry criteria that study participant should be asymptomatic at the time of study entry.

Table 1. Assessment of viral symptom severity			**Response**			
			None	**Mild**	**Moderate**	**Severe**
Item	**Question**	**viral symptom**	**Score (circle)**			
1	Question: What was the severity of your [insert symptom] at its worst over the last 24 hours?"	Stuffy or runny nose	0	1	2	3
2		Sore throat	0	1	2	3
3		Shortness of breath (difficulty breathing)	0	1	2	3
4		Cough	0	1	2	3
5		Low energy or tiredness	0	1	2	3
6		Muscle or body aches	0	1	2	3
7		Headache	0	1	2	3
8		Chills or shivering	0	1	2	3
9		Feeling hot or feverish	0	1	2	3
10		Nausea (feeling like you wanted to throw up)	0	1	2	3
			Response			
			0	**1-2**	**3-4**	**≥5**
Item	**Question**	**viral symptom**	**Score (circle)**			
11	In the last 24 hours how many times did you	**vomit (throw up)**?	0	1	2	3
12		have **diarrhea (loose or watery stools)**?	0	1	2	3
			Response			

			The same as usual	Less than usual	No sense	
Item	**Question**	**COVID-19 symptom**	**Score (circle)**			
13	In the last **24**	**smell**	0	1	2	
14	**hours** rate your sense of	**taste**	0	1	2	

Additional COVID-19-Related Assessments

In addition to assessment of key virus-related symptoms, additional virus-related assessments will be included as shown in **Table 2.**

Table 2 Additional COVID-19-Related Assessments

COVID-19-related assessments	**Comments**
Use of any medications to treat some of the COVID-19-related symptoms	List the name of type (e.g., analgesics, antipyretics) and name of medication, dose, dosage form, and date and time(s) of administration
Body temperature: the timing and the route of body temperature assessment method (e.g., oral)	Use thermometers provided to trial subjects

Oxygen saturation	Collected with specific pulse oximetry equipment provided to subjects. Investigators will provide instructions how to use.
Patient-reported global impression items assessing a) return to usual health; b) return to usual activities; and c) overall COVID-19-related symptoms:	Questions used: • In the past 24 hours, have you returned to your usual health (before your COVID-19 illness)? Yes or No • In the past 24 hours, have you returned to your usual activities (before your COVID-19 illness)? Yes or No • In the past 24 hours, what was the severity of your overall COVID-19-related symptoms at their worst? None, Mild, Moderate, or Severe

Participant timeline

Study Outline/Schedule of events/ Participant Timeline: Potential participants are screened for study eligibility by telephone ± video link; consenting individuals undergo a remote (via video-link or, if not feasible, by telephone) baseline visit (day 1) within 24 h. Subsequent remote visits occur on days 1, 7, 14. The full schedule of events is shown in **Table 4.**

Timepoints (D0 is the day of study entry)	**Before (<) D0 or D0-D4**	D1	D7	D14	D21
Eligibility assessment	X				
Informed consent	X				

Dispensation of study drug (Mito-MES arm)	X				
Assessment by study staff		X	X	X	X
Drug/no drug treatment					
Adherence assessment		Daily D1-14			
Symptoms assessment		Daily D1-14			
Temperature assessment		Daily D1-14			
Concomitant medication assessment					
Self-collected Nasal swab (PCR/rapid antigen test)	X	Daily D1-D21			
Adverse event assessment			X	X	X

Sessions by study staff

There are a total of 5 study visits over a period of 14 days. Day 0 is the day of exposure to SARS-CoV-2.

Session 1 (before D0): Screening and enrollment. A medical history, study questionnaire, eligibility assessment, informed consent will be performed by the investigator

Session 2 (before D0 or days 0-4): Dispension (allocation) of mito-MES (the study team will ship overnight the compound to the study participants).

Session 3 (day 1): Telephone consult. Medication use, history (review of systems), adverse event assessment, adherence diary, symptom diary, temperature diary will be assessed.

Session 4 (day 7): Telephone consult. Medication use, history (review of systems), adverse event assessment, adherence diary, symptom diary, temperature diary will be assessed.

Session 5 (day 14): Telephone consult. Medication use, history (review of systems), adverse event assessment, adherence diary, symptom diary, temperature diary will be assessed.

Sessions by study participant

Sessions by study participant

Day 1-21: Performance of diagnostic SARS-CoV-2 test in nasal swab

Daily on D1-14

- Adherence diary
- Symptom diary
- Temperature diary

Symptom severity scores will be recorded on days 0, 1, 7, 14 by history.

All study visits will be remote (phone call or video visit).

Screening

The following parameters will be reviewed:

- HIV status
- Age
- Gender
- Underlying condition and comorbidities
- Medications
- Pregnancy status (if applicable)
- **Demographic characteristics:** Birth sex, gender, race, ethnicity (sex will be considered as a biological variable);

- **Behavioral risk factors:** Cigarette smoking (yes or no, pack years), alcohol use;
- **Clinical factors:** Body-mass index (BMI), hypertension (based on self-report and medications), diabetes (based on self-report and medications), kidney disease (self-report), liver disease (self-report and medications), adjudicated myocardial infarction (MI)(self-report and medications), and hepatitis C virus (self-report and medications), use of hormones (e.g. testosterone or contraceptives)(self-report and medications), menopausal status (self-report);

If the participant had no available screening labs within 3 months prior to the screening visit then a history of the above parameters will be used to establish underlying comorbidities. A laboratory confirmation will not be performed. If the possible study participant is female and sexually active and there is a possibility based on history (timing of most recent sexual intercourse and timing of menstrual period) that the possible study participant is pregnant, then the participant will be excluded from the study.

Assessment of safety

1. RISKS TO HUMAN SUBJECTS

Risks related to all study participants.

We see no psychological, social, or legal risks beyond those of participation in health-related research in general. The potential physical risks of participating in the proposed experiments are described in detail below. The procedures have been used previously by the sponsors' groups without complication. Experienced investigators (Kelesidis) will provide medical oversight to ensure subject safety by serving as the physicians of record on this trial.

Physical risks

Risks of Mito-MES. See detailed Preclinical and Clinical data included in the IND application about safety profile of Mito-MES.

Psychological risks

Social risks – Although every effort will be made to protect the privacy and confidentiality of all participants, it is possible their involvement in the study could become known to others, resulting in social harms. For example, individuals could be identified as having COVID-19 and consequently, be treated unfairly or discriminated against by, families, friends and/or communities.

Loss of confidentiality risks – Although every effort will be made to protect the privacy and confidentiality of all participants, it is possible their involvement in the study, could become known to others. All guidelines for the protection of Personal Health Identifiers will be followed.

Financial risks

Financial risks– There are no financial risks associated with participation in this study. The drug will be given to study participants free of charge. In the rare event that medical care is needed during the study for serious adverse effects related to use of MitoQ the insurance of the participants may be billed and copays and deductibles may apply.

Other risks

Genetic testing – This is project includes no genetic testing.

Research subjects' alternative to participation – The research is voluntary and subjects can decide not to participate at any time during the study.

2. ADEQUACY OF PROTECTION AGAINST RISKS

a. Recruitment and Informed Consent

Recruitment and retention:

Project site:

UCLA will be the only site to enroll human subject participants. To that end, the UCLA IRB, operating under FWA 00004642, will be the IRB of record for this study. Study protocols and informed consent documents will be reviewed and approved by the UCLA Institutional Review Board (FWA 00004642) prior to research implementation. Subsequent to the initial review and approval, UCLA IRB will review the protocol at least annually. The investigators will submit enrollment, safety, and progress reports as required for annual reviews. Any amendments to the protocols will be reviewed and approved by the IRB prior to implementation.

Study site: UCLA Clinical and Translational Research Center: 10833 Le Conte Avenue, BE-144 Center of Health Sciences, Los Angeles, CA 90095

Collection process for research specimens and data: Personal data will be collected by interview in person or over the phone.

Linkages to subjects and access to subject identifiers: All study data collection and administrative forms will be identified only by a coded study ID in order to maintain participant confidentiality. Study-related documents and information collected from study participants will be stored in locked file cabinets in areas with access limited to study staff and/or on a private password-protected computer drive. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All databases will be secured with password-protected access systems. No participant's study information will be released without the written permission of the participant, except as necessary for monitoring by the NIH and/or its contractors, UCLA IRB, or monitoring bodies of the US government and regulatory agencies. Thus, all patient samples and information will be stored under a code without any other identifiers, and the PI will maintain patient identifying and contact information in a locked cabinet in a secure office.

b. Protection against Risks

Overview: The potential general risks of the proposed study also will be minimized by:

- allowing only healthy (free from chronic disease) men and women to participate;
- screening for adverse events and allergies to drugs;
- using only safe, well-established procedures with only qualified and experienced personnel performing the procedures;
- ensuring constant personal monitoring of each experimental session by the investigators and staff;
- employing record keeping processes with complete confidentiality;
- keeping all records in safe, locked facilities on the campus;
- not associating individual subject data with subject name (all stored data will be coded).

More specifically protection against specific risks includes:

Good Clinical Practice – Guidelines will be in place to maintain participants' confidentiality with regard to health status during clinical investigations, laboratory testing of specimens, and medical records documentation (see "*Linkages to subjects and access to subject identifiers*" in section 1b). Data collection forms and study specimens will include unique study numbers only. The code sheet linking subjects' identification data, such as names and medical record numbers, with the study ID number, will be kept in a locked cabinet in the study research office, with access limited to the designated study staff. Computer data will be stored only by study ID numbers. Any published results from the study will appear as tabular descriptions of study groups. These processes have proved successful in our previous research projects. To limit potential risks of medical procedures, only licensed, trained and experienced staff will conduct research procedures.

Study Discontinuation. The study may be discontinued at any time by the IRB, OHRP, or other government agencies when necessary to ensure the protection of research subjects.

Protection of Health Information. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB or OHRP. Local regulatory rules will be followed, and are in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA) to protect the health information of individuals obtaining healthcare in the USA. Subjects will be provided with a document explaining HIPAA at the time of obtaining informed consent.

Confidentiality. Good Clinical Practice guidelines will be in place to maintain participants'

confidentiality with regard to health status during clinical investigations, laboratory testing of specimens, and medical records documentation. Data collection forms and study specimens will include study numbers only. The code sheet with subjects' identification data and study numbers will be filed and kept in a locked cabinet in the study research office with access limited only to study staff. To ensure security of computer-stored information, data will be stored by identification number only. These processes have proved successful in multiple research projects at this site.

Vulnerable Populations. Women who are not pregnant and children will be allowed to participate in this study.

Risk for pregnancy: Since the study drug (mito-MES) has not been studied in pregnant women, women of child bearing potential should use a barrier method that prevents pregnancy in combination with a highly effective method of contraception. Highly effective methods are:

- oral contraceptive
- Use of condoms during any sexual intercourse during the study period
- intrauterine device
- intrauterine hormonal releasing systems
- Bilateral tubal ligation/occlusion
- Vasectomized partner provided that partner is the sole sexual partner of the participant and that the vasectomized partner has received medical assessment confirming surgical success.

Since the effects of mito-MES on sperm cells have not been established in humans, men of child bearing potential should use a barrier method that prevents pregnancy during any sexual encounter during the study period. Highly effective methods for men are:

- Use of condoms during any sexual intercourse during the study period

• Vasectomized study participant who has received medical assessment confirming surgical success.

Minimizing Specific Risks Related to therapy: Risks of oral Mito-MES treatment will be minimized by:

- careful telephone screening of subjects;
- conducting all study activities in a safe, supervised clinical research setting;
- monitoring of the status of all subjects throughout the protocol by the staff and physician of record, Dr. Kelesidis;
- using a dose and duration of Mito-MES treatment shown to be safe and well tolerated in humans;
- having subjects consume Mito-MES with food to minimize gastrointestinal discomfort.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

For most of the subjects who participate in this study there are no direct benefits. Based on our preliminary data it is possible that our proposed interventions may prevent development of severe viral disease and this will benefit the participant. However, there may be personal satisfaction from the fact they are aiding in important medical research and that the knowledge obtained from this study will advance potential treatments for viral infections including COVID-19. The risks to individual participants are outweighed by potential benefits to the scientific community and to society – namely, extensive contribution to the medical knowledge bank. The risks are relatively minor. Adequate protections against potential risks are built into the research project.

DATA AND SAFETY MONITORING PLAN

DATA AND SAFETY MONITORING PLAN

a) Medical Supervision and Subject Surveillance. Dr. Theodoros Kelesidis, board certified internist and infectious diseases/HIV specialists, will serve as the Physicians of Record for the study and has extensive experience with clinical oversight of such trials. Dr. Kelesidis will make final decisions regarding subject screening/enrollment and monitor/oversee clinical status and subject safety. All of the screening data and information collected throughout the study will be evaluated by Dr. Kelesidis.

b) Data Safety Monitoring Plan. To ensure the safety of subjects, they will need to meet rigorous inclusion/ exclusion criteria, including comprehensive health screening procedures. The participants' personal physicians will also be informed of their patients' participation in the study.

Supervising Physician. The local investigator (Kelesidis) will have access to all data related to safety assessments. Safety will be monitored via questionnaire. Safety assessments will be performed at study visits. At the screening visit, after review of eligibility, subjects will provide a detailed clinical history (including smoking, alcohol and medication history). Subjects will then undergo a full clinical systems review.

The local investigator (Kelesidis) will be available to provide direct evaluation of any clinically-related concerns that might arise during the study. The principal investigator (Kelesidis) will directly discuss with the members of UCLA IRB any concerns for adverse events and make recommendations for continuing or stopping the study. Given published safety data of Mito-MES we do not anticipate any major clinical side effects related to safety of Mito-MES especially since every effort will be made to minimize any potential side effects such as nausea

(administration of <80 mg of Mito-MES, administration with a full stomach, exclusion of participants with known gastrointestinal disease that may predispose them to nausea).

Interpretation of Results/Statistical Analyses:

There is no formal hypothesis testing in this exploratory study and a comparison with a dedicated control group. Observational and descriptive data related to efficacy and safety of use of MitoQ for viral infections will be obtained and these data will set the foundation for larger controlled clinical trials. All categorical and continuous data (outlined above) will be recorded as median and interquartile range (given small sample size). Exploratory analyses will be conducted by parametric or non-parametric tests for continuous or categorical parameters as appropriate to explore whether the primary outcomes defer between men and women. The severity of symptoms will be compared first by categorical analysis (symptoms present yes or no) via Fisher's exact Chi square, and subsequently via the independent two-sample t test for symptom severity among those who are symptomatic. Non-normally distributed data will be analyzed via the Mann–Whitney U test. Females may have differences in mitochondria compared to males of identical age. We will conduct secondary analyses to explore if effects of MitoQ will vary by sex and race; however, a fully powered, sex- and race-stratified analysis is beyond the scope of this study.

Sensitivity analyses: Sensitivity analyses will be performed based on established guidelines regarding the performance of sensitivity analyses in clinical trials[92,93]. Intention-to-treat analysis (as primary analysis), As-treated analysis, Per-protocol analysis will be performed to assess non-compliance or protocol violation. Analyses will be performed with and without outliers as

assessed by z-score or boxplot. The primary analysis will be compared with the analysis that ignores clustering with one primary method chosen to account for clustering. Analysis will be performed with and without adjustment for baseline characteristics. Finally, analyses will be performed under different distributional assumptions, if applicable.

Adjustments for multiple comparisons: Notably, the proposed study is exploratory and there is less need for a multiple-testing correction, as any false-positive findings will not change practice. Any findings will be tested in further confirmatory clinical trials.

Missing data

Investigators will make efforts to minimize the amount of missing data including providing reminders (e.g., phone calls, text messages, email) to trial subjects to complete PRO instruments, monitoring compliance with PRO instrument completion throughout the assessment period, following up with trial subjects who are not successfully completing PRO instruments (and, where permissible, close contacts if the trial subject is not responding), and recording verbal responses for those who are unable to self-record because of illness or other circumstances. Investigators will obtain contact information for close contacts of trial subjects for use in case of nonresponse. If the investigator or a member of the study team plans to contact a family member or other close contact when subjects do not respond to follow-up, this will be described in the informed consent document approved by the institutional review board. The reasons for missing data will be documented. The informed consent process and informed consent document will include information to educate prospective subjects about the continued scientific importance of their follow-up data even if they choose to discontinue treatment. The investigators will plan appropriate methods for handling missing data in the analyses, considering the reason for the missing data based on established guidelines[94].

The investigators will prospectively specify how hospitalization will be handled in the statistical analysis. If hospitalization occurs in a study participant, the investigators will determine whether the hospitalization is associated with the viral infection or whether the hospitalization was irrelevant. If the hospitalization is associated with the viral infection then the participant would almost always become symptomatic before any hospitalization and hospitalization would not result in missing data with regards to the primary outcome. The hospitalization will not be considered a form of missing data in this case. If the hospitalization is not associated with viral infection such as SARS-CoV-2 infection and study participant was asymptomatic at the time of death, then the investigators will consider the specific datapoints as “missing data” that will be determined as outlined in this section.

The investigators will prospectively specify how death will be handled in the statistical analysis. If death occurs in a study participant, the investigators will determine whether the death is associated with viral infection such as SARS-CoV-2 infection or whether the death was irrelevant. If the death is associated with SARS-CoV-2 infection then the participant would almost always become symptomatic before any death and death would not result in missing data with regards to the primary outcome. The death will not be considered a form of missing data in this case. If the death is not associated with SARS-CoV-2 infection and study participant was asymptomatic at the time of death, then the investigators will consider the specific datapoints as “missing data” that will be determined as outlined in this section.

Missing data will be handled by the mixed effects models in the analysis, which assumes that the missing data mechanism is ‘missing at random’. The analysis will be redone by independent methods by analyzing only complete cases versus impute the missing data using single or multiple imputation methods. The aim of the missing data approach will be to ascertain vital status for all

trial subjects even after a subject decides to discontinue treatment or discontinue participation in the trial, including follow-up for key outcomes, while adhering to informed consent requirements.

Sample Size Considerations

Power/Sample Size:

The study is purely exploratory and is not powered to do a formal hypothesis testing. The data from this study will set the basis for large randomized controlled clinical trials to test the safety of mito-MES in the setting of treatment of viral illnesses. In addition, preliminary data will be obtained on the efficacy of Mito-MES to reduce severity of viral illness. More specifically, all the proposed primary endpoints of efficacy are based on objective quantifiable criteria (fever based on thermometer, duration of symptoms based on number of days, severity of viral illness based on quantitative summary score that considers at least two independent symptoms of viral illness based on a clinically relevant definition of viral illness made by a physician). The “effect size” of the intervention Mito-MES on these quantifiable outcomes of viral illness in humans will be determined. The safety of Mito-MES in the setting of viral illness will be recorded. This is essential information for the design of larger RCT studies.

Based on resources, we enrolled 80 study participants.