VERSION 7/27/20

TEMPLATE PROTOCOL INSTRUCTIONS:

- *Use this "TEMPLATE PROTOCOL (HRP-503)"if this is an investigator-initiated study where a protocol document has not already been provided to you with relevant information for each applicable section listed in the Table of Contents (below).*
- *Depending on the nature of the research, some sections may not be applicable. If a section is not applicable, mark as "NA" or delete altogether. For example, research involving a retrospective chart review may have many sections marked as NA or deleted.*
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- *Please note that underlined items in this document are hyperlinked to additional information.*

STUDY TITLE:

Prevention of COVID-19 Progression Through Early Administration of Inhaled Nitric Oxide. The NO-COVID Study.

STUDY SPONSOR: Marvin A. Konstam, MD

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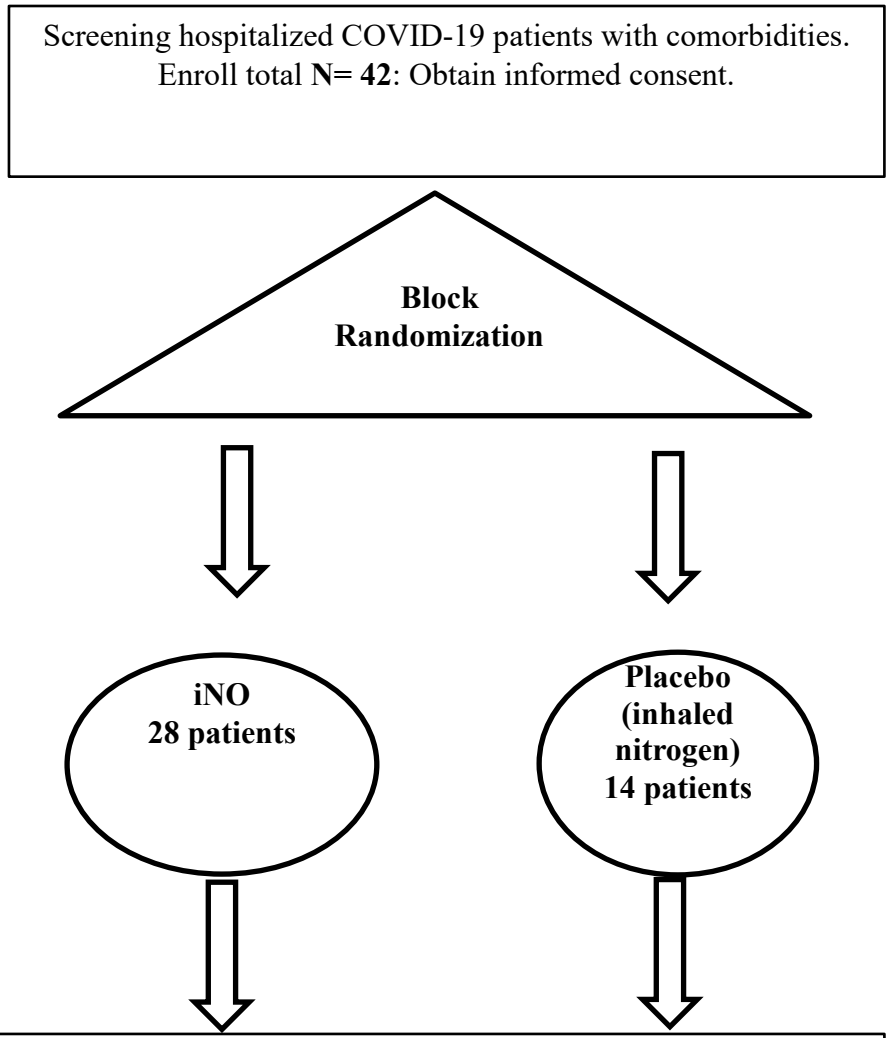
A. Study Schema



Baseline

Baseline assessments: measurement of disease severity score (Table 1), assessment of dyspnea (dyspnea score), oxygen saturation, ABG, IL6, TNFa, fibrinogen and CRP, ferritin, D-dimer; creatinine, BUN, eGFR; Pregnancy test.

Daily up to 28 days

Follow up assessments of study endpoints and safety: disease severity score (Table 1).

Assessment of dyspnea (dyspnea score) 6±2 hours after initiation of treatment/placebo and daily thereafter

Blood measurements at specific timepoints:

-24 hours: IL6, TNFa, fibrinogen*, CRP*, ferritin*, D-dimer*, and methemoglobin

-72 hours: IL6, TNFa, fibrinogen* CRP*, ferritin*, D-dimer*

-1 week: methemoglobin, IL6, TNFa, fibrinogen* CRP*, ferritin*, D-dimer*

- Daily vital signs*, creatinine*, BUN*, eGFR*

Presence or absence of inotrope use.

* Standard of care.

B. Introduction

B.1 Background and Rationale

Presently, the majority of patients testing positive for COVID-19 do not require hospitalization, have a self-limited course, and appear to have complete clinical recovery within 2-3 weeks [1]. However, 20% become hospitalized; approximately 5% of the total require critical care management; and overall mortality ranges from 1-3% [1,2]. However, progression to severe disease, intubation, and death varies with underlying risks, including advanced age. For example, mortality ranges from 3-11% for patients aged 64-85 years and 10-27% for those > 85 years old [2]. A treatment preventing disease progression in patients with mild to moderate hypoxemia, but at high risk for severe worsening, would represent a major healthcare advancement, with potential for substantial reduction in morbidity and mortality, as well as marked reduction in overall healthcare utilization and cost.

Clinical worsening in COVID-19 is characterized by progressive dyspnea, reduced oxygenation, radiographic abnormalities, often leading to a pattern consistent with acute respiratory distress syndrome (ARDS). Patients who succumb often manifest sudden progression, pressor-resistant hypotension, massive systemic release of cytokines, and inability to oxygenate, with progressive alveolar-arterial oxygen gradients. Apparent clinical differences from other causes of ARDS include the rapidity of deterioration, the prominence of cytokine release, relatively maintained lung compliance, greater responsiveness to proning, and myocardial depression (likely cytokine-mediated), and vasoplegia, resulting in shock [3,4].

The COVID-19 virus enters the lungs via the receptor angiotensin converting enzyme-2 (ACE-2) [5]. Associated functional down-regulation of ACE-2 may play a role in mediating lung injury, through imbalance between angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7), an ACE-2 cleavage product of Ang II [6]. The clinical syndrome may be mediated, at least in part, via pulmonary vasoconstriction, vascular inflammation and formation of microthrombi, increased oxidative stress, increased leukocyte and macrophage adhesion, reduced nitric oxide (NO) production and stability, endothelial dysfunction, and fibrosis.

The pattern of risk factors identified for severe COVID-19 shows some overlap with complications of other viral infections, yet appears to be unique. High risk is associated with advanced age, type-2 diabetes mellitus (T2DM), hypertension, and obesity [7,8]. These are the precise risk factors for a specific form of heart failure with preserved ejection fraction (”HFpEF”), an end-product of what has been called metabolic-inflammatory cardiovascular disease (M-I CVD) [9-11]. The likely pathobiology of the M-I CVD syndrome has been well-described, including cytokine-mediated vascular inflammation, endothelial dysfunction, reduced production of NO and cGMP and cardio-myocyte hypertrophy with interstitial fibrosis [12]. This pattern is strikingly similar to, and may be synergistic with, the likely pathologic mechanisms of severe COVID-19.

We therefore postulate that therapeutic mitigation of this biologic pattern, likely characteristic of both M-I CVD and advanced COVID-19, will prevent the clinical progression, in patients at high risk, from the early stages of acute COVID-19 to extremis and death. One of the earliest manifestations of M-I CVD is impaired production of NO by endothelial cells [13,14]. Although nitrate therapies have not proven beneficial in patients with established HFpEF, in that case, treatment was aimed at improving diastolic function in patients with established cardiac disease. These patients were no longer likely to benefit from addressing the basic mechanisms that led to the ultimate cardiac structural and functional changes.

Inhaled NO (iNO) is a potent pulmonary vasodilator, with established safety in patients with ARDS [15-17] and in those with right heart failure following cardiac transplant. It functions to maintain the anti-inflammatory and anti-thrombotic functions of the vascular endothelium. It improves oxygenation in patients with ARDS with minimal systemic effects [15], though it is not proven to improve survival in studies of established ARDS [16-17]. However, prior non-confirmatory studies of iNO in patients with ARDS enrolled a heterogeneous population, in terms of etiology and underlying disease mechanism. Also, in contrast to patients with acute COVID-19, the lung injury of these patients was associated with increased lung stiffness. Those studies also focused on patients with

advanced disease, who might not have had an opportunity for maximal benefit from the mechanisms of iNO action.

SPECIFIC AIM: To test the hypothesis that early supplementation of NO in the lung will prevent the progression of COVID-19 to fulminant ARDS by attenuating pulmonary vascular inflammation and microthrombi formation and by reversing pulmonary vasoconstriction to improve oxygenation. Thus, we intend to investigate the protective role of inhaled NO in hospitalized COVID-19 patients with early systemic oxygen desaturation and risk factors for progression. We propose a randomized, open-label pilot study of 42 hospitalized, higher-risk COVID-19+ patients, with mild to moderate systemic oxygen desaturation, with the goal of preventing worsening oxygenation and need for increasing oxygen supplementation and/or ventilatory support. Based on the results of this study, we intend to proceed with a large scale investigation to definitively assess the impact on major clinical outcomes. Our presently [18] proposed trial is unique in 1) focusing on prevention of progression in patients before they become severely ill and 2) aiming to provide clinically valuable data within the next 3 months.

B.2 Risks to Subjects

Inhaled Nitric Oxide is generally well tolerated without significant side effects. Adverse hemodynamic effects such as hypotension are very brief and are immediately reversible after discontinuation of the iNO due to the short half-life. Methemoglobinemia is a rare occurrence at the dose we are administering. NO2 monitoring is not available on the INOPulse device and is not deemed necessary because at the dose to be used (125 mcg/kgIBW/min, corresponding approximately to 20 ppm), the level of NO2 generated is far below potentially toxic levels (18). Finally, iNO administration carries a small risk of renal dysfunction. [19]

Patients randomized to the placebo group will receive inhaled nitrogen (N2, 99.999%) gas. Nitrogen is a colorless, odorless gas that makes up to 78% of the air we breathe. Since nitrogen is part of the atmospheric air, inhalation of nitrogen is well tolerated without side effects.

Physical risks associated with the blood draw include bruising and/or some bleeding around the catheter and, rarely, infection. Lightheadedness and fainting may result from the blood sampling procedure.

Non-physical risks to subjects include breach of privacy, all patient information will be de-identified and coded to mitigate this risk. No PHI will be retained for the purposes of this study. A unique, numeric code will be assigned to each patient. The log linking the numeric code to the patient's health information will be maintained on a secure, password-protected computer in a locked research room. Only study personnel will have access to the log.

The study investigators and the staff performing this study are at risk of being infected by SARS-COV2 and developing COVID-19. In order to minimize the risk, the investigators will use personal protective equipment (PPE) according to the Tufts Medical Center guidelines. We will also implement the remote consent process. This process includes virtual meeting via Zoom or Facetime among the investigator, the research team and the participant. Once the ICF has been reviewed and all questions have been answered, DocuSign app will be used to acquire electronic signatures. A copy of the ICF will be printed and given to the participant. Another copy will be printed and given to the unit coordinator to put in the patient’s medical records. The team will also create a template note that will be added to the patient record in Soarian. The note will document how consent was done and with whom.

B.3 Potential Benefits to Subjects

Although there, may be benefit from iNO, there is presently equipoise around this question. Therefore, there will be no direct benefits to the study subjects. However, successful completion of this study will provide significant scientific information on the effect of a relatively safe and readily available agent (iNO) on the prevention of COVID-19 progression.

B.4 Alternatives

Participation in this study is completely voluntary. Patients can decide to participate or not to participate. Instead of being in this research study, treatment choices include standard supportive care with oxygen supplementation as needed based on the severity of your clinical presentation. Patients participating in this trial will not be prohibited from participating in any other trial and will be able to receive any other therapy deemed appropriate by the care team. We will exclude patients already receiving iNO. However, if, in the unusual event that the clinician determines that a study patient requires iNO, he/she may discontinue study drug (without breaking the blind) and initiate open label iNO. The patient will, nevertheless, remain in the trial, with appropriate collection of endpoints.

C. Objectives

Our primary objective is to investigate the hypothesis that inhaled NO will reduce clinical worsening of hospitalized, high-risk patients with early COVID-19 to progressive systemic de-oxygenation, intubation, and death.

Our secondary objective is to investigate the hypothesis that the beneficial effects of inhaled NO occur coincident with a decrease in systemic inflammation in COVID-19.

D. Enrollment and Withdrawal

D.1 Inclusion Criteria

1. Age 18-90 years.
2. Admitted to the hospital (med-surg or critical care) with dyspnea
3. Diagnosis of COVID-19 based on either
   a) positive nasal or oral pharyngeal swab by PCR, or
   b) highly probable clinical picture based on clinical and CXR/CT scan
4. Requiring oxygen supplementation OR O_2 saturation on room air of $\leq 94\%$
5. At least 1 of the following 4 risk factors for clinical worsening:
   a) Age ≥ 60 years
   b) T2DM or pre-diabetes as evidenced by either treatment with a hypoglycemic agent or any documented HgA1c ≥ 5.6
   c) Obesity, based on BMI ≥ 30 kg/m2
   d) Hypertension, based on treatment with an antihypertensive medication or systolic or diastolic blood pressure measurement ≥ 140 or ≥ 90 mmHg, documented at enrollment or at any time within the prior 6 months.

D.2 Exclusion Criteria

A subject is not eligible for inclusion if any of the following criteria apply:

1. Intubated or prior intubation (during present hospitalization) or anticipated intubation within the subsequent 2 hours.
2. Receiving > 5L/min flow nasal O_2 to maintain O2sat $\geq 92\%$
3. Using high-flow nasal cannula (HFNC) or non-invasive ventilation (NIV)
4. Receiving iNO, a PDE5 inhibitor, oral or intravenous nitrates, nitroprusside, prilocaine, sulfonamides, or riociquat.
5. Other major pulmonary, cardiac, such as chronic obstructive lung disease or heart failure, or systemic illness or disease involvement with potential to represent the primary driver for clinical deterioration within the next 3 days.
6. History of group 1 pulmonary hypertension.
7. Pregnancy
8. Active breast feeding
9. Acute kidney injury (AKI), evidenced by acute doubling of serum creatinine within previous 48 hours

10. Clinically relevant spontaneous alteration of mental state
11. Inability for the patient (or proxy) to provide written informed consent.

Eligibility will be assessed after reviewing medical records, obtaining medical history and performing physical exam.

Eligibility will be determined by the PI or the Co-I.

Study subjects can participate in another research study while participating in this research study.

D.3 Withdrawal of Subjects

Subjects may withdraw at any time. Data collected up to the point of withdrawal will be retained in study documents.

D.4 Recruitment and Retention

D.4.1 Local Recruitment Methods

PI and Co-Is will identify potential study candidates from the COVID-19 positive patients who are admitted at Tufts Medical Center. A HIPAA waiver for screening is requested to identify potential subjects. No recruitment materials will be given to potential subjects. No identifiable data will be retained for subjects that are determined to be ineligible.

D.4.2 Study-Wide Recruitment Methods

Is this a multicenter study where subjects will be recruited by methods not under the control of the local Tufts site (e.g., call centers, national advertisements)?

☐ **Yes** ☑ **No**

D.4.3 Payment

Will subjects receive money, gifts, or any other incentive for participating in this study?
This does not include reimbursement for expenses, which is considered in the next section.

☐ **Yes** ☑ **No**

D.4.4 Reimbursement

Will subjects be reimbursed for their expenses, such as travel, parking, meals, or any other study related costs?

☐ **Yes** ☑ **No**

E. Costs to Subjects

Does the research involve any costs to subjects?

☐ **Yes** ☑ **No**

F. Study Design

F.1 Study Timelines

From April 2020 through April 2021 we plan to enroll 42 hospitalized patients with COVID-19, at risk for worsening, based on baseline systemic oxygenation and at least one of the major risk factors of age ≥ 60 years, type II DM, hypertension, and obesity. The patient will be followed, and clinical stage determined

daily, through discharge, death or 28 days post-randomization. Study drug will be given for up to 2 weeks unless the patient deteriorates and requires escalation to high flow oxygen or intubation or improves and is no longer deemed to need therapy. If the study drug is discontinued at 2 weeks or because of improvement and the patient subsequently deteriorates, the study drug may be resumed.

F.2 Procedures

Eligible subjects will be identified by an investigator or study coordinator and screened by a study coordinator. A HIPAA waiver for screening is requested to identify potential subjects. Subjects will be approached for informed consent and, upon consenting (see details about the remote consent process below), the following procedures will be followed. Pregnancy will be ruled out in women of child-bearing age with a pregnancy test prior to randomization.

Randomization and iNO administration:

We will perform computerized block randomization (on day zero) with a 2:1 study drug-to-placebo ratio to receive blindly either pulsed inhaled nitric oxide, in addition to standard of care, or inhaled nitrogen gas (N2, 99.999%) in addition to standard of care. Randomization will be stratified by being in clinical severity stage 1 or stage 2 (see Table 1, below). Randomization will occur in blocks of 6 subjects: 4 iNO and 2 placebo. Subjects will receive iNO using the INO pulse device at a dose of 125 mcg/kg IBW/hr (equivalent to approximately 20 ppm). The INO pulse device will also be used for administration of inhaled nitrogen as placebo at a dose of 125 mcg/kg IBW/hr. The randomization will be performed by the research pharmacy as described below.

The device:

- is the size of a paperback book and weighs approximately 2.5 pounds
- has a battery life of approximately 16 hours when fully charged
- has a simple and intuitive interface that is user friendly
- is compatible with many long-term oxygen therapy systems that operate via a nasal cannula
- ensures a steady and accurate dose is administered by automatically adjusting to a patient's breathing pattern.

The clinical disease severity will be assessed pre-randomization as the worse of 2 scores measured 2 hours apart. Patients eligible for randomization will be those with scores of 1 or 2 (below), and randomization will be stratified according to score (1 or 2). Study drug will begin within 1 hour of randomization. Beginning on the day following randomization ("day 1"), we will calculate clinical score, daily, as the average of 3 measurements taken within 2 hour windows centered at 6AM, 2PM, and 10PM according to the following table:

TABLE 1

The following severity score 3 times daily, based on the level of oxygenation / ventilation support, where the treatment target is 92% ≤ O_2 saturation < 96%:

Stage	Oxygen support
0	Not receiving O2 supplementation; AND room air O2 saturation ≥95%
1	Supplemental O2 ≤ 2 liters/min; OR room air O2 saturation ≤ 94%
2	Supplemental nasal O2 >2 and ≤ 5 liters/min
3	Supplemental nasal O2 >5 liters/min
4	HFNC or NIV with FiO2 > 50%
5	Intubation, ECMO, or need to intubate with "Do not intubate" order
6	Death

HFNC = high-flow nasal cannula; NIV = non-invasive ventilation

The primary endpoint is the maximum daily disease severity score recorded from day 1 through discharge, death or 28 days post-randomization. We will test the difference in the average maximum disease severity score between the two treatment groups (see analysis section below).

Experimental Procedures:

Venous blood samples will be drawn as described in paragraph F3.

Clinical Data Collection

- Medical record will be reviewed for medical history, admission characteristics, diagnostic values, hemodynamic data, and other relevant data.
- Clinical characteristics including age, date of birth, gender, BMI, comorbidities (such as hypertension, diabetes, history of cardiovascular disease) will be collected from medical records.
- Baseline vital signs will be collected from chart review.
- Clinically indicated lab results will be collected from medical records.

Our study has a control arm. Patients randomized in the control arm will receive inhaled nitrogen gas (placebo) in addition to standard of care as opposed to iNO plus standard of care, in a double blinded fashion. The research pharmacy will be responsible to randomize the patient and dispense the study drug in a blinded fashion. The active study drug, iNO, will be supplied by the drug company to the research pharmacy in size 0.074 liter aluminum cartridges at a concentration of 6.0 mg/L (4880 ppm). Placebo to match study drug will also be supplied by the drug company to the research pharmacy in size 0.074 liter aluminum cartridges containing nitrogen (N2, 99.999%) gas.

Both the nitric oxide and placebo cartridges will be labeled identically to maintain the blind throughout the study. All study drug kits and cartridges will be labeled with informative language that each cartridge contains up to 6.0 mg/L (4880 ppm) NO or nitrogen gas to allow for compliance with compressed gas transportation regulations while maintaining a blinded label. Prior to shipment to the investigational site, all study drug will be labeled with unique identification numbers that will be used to trace the units to the specific treatment type and subject. Drug kits and cartridges are intended for use by a single subject.

The research pharmacy staff will serve as the unblinded personnel. They will be responsible for securing the list of unique numbers and their corresponding treatment types (6.0mg/L (4880ppm) NO gas or matching N (placebo), as well as assigning the specific kit and cartridge numbers that correspond to the treatment assignment of each individual subject.

The blind may be broken only if specific urgent treatment would be dictated by knowing the treatment status of the subject. In such cases, the Investigator or designee will contact the unblinded team member to request the subject treatment be unblinded. The date, time, and reason for the unblinding will be documented.

Because of the significant limitations of making clinical assessments on COVID-19 positive subjects we will use the oxygen saturation and oxygen support recorded by nursing as part of routine care. The recorded oxygen support and oxygen saturation will be verified by the research personnel without the need for research personnel to use personal protective equipment.

Dyspnea score endpoint. Dyspnea will be assessed using a visual analog scale from 0 (not at all) to 10 (very severe). Patients will be asked “How short of breath are you?” and directed to indicate their response on the scale. They will mark their response with ink, which will be timed and enables patients to track their responses. The scoring sheet will be photographed during a routine clinical visit, using a secure iPad, with the

image transformed into a pdf to maintain documentation. Dyspnea ratings will be done at baseline, after 6±2 hrs of therapy and daily thereafter up to a week or as long as therapy continues. This score has been described in prior studies by Dr Hill. There is no added risk to the patient. (Kramer N, Meyer TJ, Meharg J, Cece RD, Hill NS. Randomized, prospective trial of noninvasive positive pressure ventilation in acute respiratory failure. Am J Respir Crit Care Med. 1995 Jun;151(6):1799-806.)

F.3 Evaluations

Will you perform any laboratory tests for this study?

☑ **Yes** ☐ **No**

If **Yes**, describe the following

- As is routine for standard of care, blood tests for fibrinogen, CRP, ferritin, D-dimer levels will be measured at baseline, 24 hours, 72 hours, and one week. These will be processed through the clinical pathology laboratory, and in compliance with Clinical Laboratory Improvement Amendments (CLIA) of 1988
- An extra tube of blood will be drawn at baseline, 24 hours, 72 hours, and one week, and analyzed in the MCRI laboratory for the measurement of IL6 and TNFa for research purposes only. (See Section F4) An extra tube of blood will be drawn at 24 hours and at one week and processed through clinical pathology for the measurement of methemoglobin if patients are still receiving iNO or inhaled nitrogen gas at these time points.
- Blood draw for these additional tests will be timed to coincide with those done for routine clinical care so as to avoid additional blood draws.

F.4 Collection and Storage of Human Biological Specimens (Tissue Banking)

Will biological specimens be stored for **future, unspecified**, research?

☑ **Yes** ☐ **No**

Venous blood will be collected from the patient prior to iNO initiation and at 24 hours, 72 hours and 1 week after iNO initiation. One venous blood sample will be placed into an EDTA-treated vacutainer tube and the second sample will be placed into a vacutainer tube without anticoagulant. The tubes will be immediately placed on ice and transferred to the laboratory for processing. The blood samples will be centrifuged for 12 minutes at 1500 x g at 4°C. After centrifugation, the supernatant from the EDTA treated tube (plasma) and the supernatant from the vacutainer tube without anticoagulant (serum) will be transferred to microcentrifuge tubes using a disposable bulb pipette. The microcentrifuge tubes containing the plasma and the serum will be stored in a -80°C freezer in the MCRI for at least 2 years. The plasma and the serum will be used for the investigational measurement of IL6, TNFa and future research investigations. Samples will be labeled with the patient's study ID and may be released to outside investigators.

G. Ethics and Protection of Human Subjects

G.1 Informed Consent Process

Will subjects be required to provide informed consent? Yes, and non-English speakers will be permitted to enroll using interpreters and IRB approved Short Forms

☑ **Yes** ☐ **No**

We will follow the remote consenting process in order to minimize the risk of viral transmission to the research staff and the patient's legal representatives:

After we identify the potential participant (or the legal representative if the potential participant is unable to consent) we will set up a time for a Zoom or Facetime call with an investigator (PI or Co-I), the participant, an impartial witness (not on the study team), and a study coordinator. The investigator will review the entire ICF with the participant (or legal representative) via Zoom or Facetime. If an interpreter is needed, they can be on the call, or a family member can interpret. Everyone on the phone call listens to the ICF review

and has an electronic copy in front of them. Once the ICF has been reviewed and all questions answered, DocuSign app will be used to acquire electronic signatures. Specifically, through DocuSign, the ICF will be sent first to the participant (or legal representative), then to the PI. Once all electronic signatures are acquired, a copy with be saved at the study files. Another copy will be printed and given to participant (or legal representative) to keep. Finally, a third hard copy will be printed and given to unit coordinator to put in the patient´s medical records. They research team will also use a remote consent verification document to verify how consent was given by the participant or their legal representative. The document will be placed into the electronic medical records and electronically signed and time stamped by the investigator. It will serve to document the process of obtaining consent.

If the potential participant is unable to electronically sign the document, the following process will be used. A witness, who is not a member of the study team, will be present on the call explaining the ICF to the participant or their legal representative. The witness will be present on the call when the participant consents to join the study. The witness will then electronically sign the ICF document using DocuSign. The research team will also use the remote consent verification document to verify how consent was given by the participant (or legally authorized representative) and the witness who was present. The document will be signed and dated by the PI or Co-I. The time will also be documented. A hardcopy of the electronically signed ICF will be printed and given to the participant. A copy will also be filed electronically in the patient's medical record along with the consent verification document.

G.2 Waiver or Alteration of Consent Process

This applies for studies where informed consent will not be obtained, required information will not be disclosed, or the research involves deception.

1. Is a waiver or alteration of the consent process being requested for this study?
   ☐ **Yes** ☑ **No**

2. Is a waiver of the consent process being requested for parents for research involving children?
   ☐ **Yes** ☑ **No**

3. Is a waiver of the consent process for planned emergency research being requested?
   ☐ **Yes** ☑ **No**

G.3 International Research

Refer to the IRB's International Checklist and International Guidance and include all relevant information described in those documents in this protocol: N/A

G.4 Confidentiality

Procedures to protect subject confidentiality:

All study documentation will be stored in the PI's locked office. All subjects will be assigned study ID numbers and wherever possible all data will be marked with the study ID number instead of the name or personal identifier. Any research material sent to the Sponsor will be redacted and de identified. All study records will be retained for the timeframe described in the record retention policy of the "SOP – Records Retention Timeframe – Investigators." Any research information that is part of the subject's medical records will be kept indefinitely. All paper research records will be kept on campus at Tufts Medical Center, de identified and in a locked office.

How data will be coded, recorded, and stored to protect confidentiality:

Subjects will be identified on the Case Report Form (CRF), documents where study-related data will be collected, by an identification number. The investigator will maintain a confidential list of the subjects involved in the study, separate from the CRFs that will serve as a means of linking the subject identification number to the medical records. All data used in analysis and reports will be used without identifiable reference to the patient.

Only the PI and study coordinators will have access to the code key for the CRF.

PI, Co-Is, Research Coordinators, will have access to study records.

We confirm that we will follow the "Confidentiality and Data Security Guidelines for Electronic Research Data" for electronic data

G.5 Screening Data Collection Form/Screening Log

This section specifically refers to data collected about potential subjects who are screened, but have not signed consent, for example potential subjects whose medical record is reviewed to see if they are potentially eligible, potential subjects who respond to a telephone screening call where the research team records information about the potential subject, etc. In this section "Screening Data / Screening Log" refers to any form of data collection on potential subjects who have not yet signed consent. For more information, refer to https://privacyruleandresearch.nih.gov/clin_research.asp.

Will a screening data/screening log be used in this research study?

☐ **Yes** ☑ **No**

G.6 Provisions to Protect the Privacy Interests of Subjects

In order to protect the privacy interests of the study subjects we will ensure that discussion of the study will take place in a private area where subjects cannot be overheard. We will also make sure that the study subjects are comfortable with the research team members.

G.7 Provisions to Monitor the Study to Ensure the Safety of Subjects

Inhaled NO is a colorless, odorless gas that offers a substantial safety margin at the doses contemplated. It is rapidly deactivated by combination with hemoglobin upon entry into the systemic circulation. Therefore, it is unlikely to lower blood pressure or cause systemic side effects. An excess of methemoglobin is exceedingly unlikely with doses being administrated. Renal impairment caused by iNO appears to be rare.

The principal investigator, with the assistance of the study coordinator, will be reviewing the safety data at least monthly. Additional safety monitoring will be performed by a physician who otherwise has no involvement in the study. The Data Monitoring Committee will meet to monitor unblinded safety data after the first 20 participants. Blinded SAEs will be reviewed weekly. The data will be obtained from chart review and from the study database.
By protocol, we will discontinue iNO if the patient needs to escalate care to HFNC, NIV, or intubation. In addition, we will discontinue iNO if:

- there is any suspicion that iNO is leading to harm and, specifically if:
- the patient develops sustained hypotension with systolic blood pressure < 85mmHg, unresponsive to fluids;
- the patient develops AKI, evidenced by a doubling of serum creatinine compared to baseline;
- methemoglobin level exceeds 5%

The inhaled nitrogen is a colorless, odorless gas that makes up to 78% of the air we breathe and does not impose any risk on the patient.

G.8 Vulnerable Populations

If the research involves individuals who are vulnerable to coercion or undue influence, describe the rationale for their inclusion and the additional safeguards included to protect their rights and welfare.

1. Can or will pregnant women be enrolled?

   ☐ **Yes** ☑ **No**

2. Can or will the research involve neonates of uncertain viability or non-viable neonates?

   ☐ **Yes** ☑ **No**

3. Can or will subjects who are not yet adults (neonates, children, teenagers) be enrolled?

   ☐ **Yes** ☑ **No**

4. Can or will minors who are:
   - i) married, widowed, divorced; or
   - ii) the parent of a child; or
   - iii) a member of any of the armed forces; or
   - iv) pregnant or believes herself to be pregnant; or
   - v) living separate and apart from his/her parent or legal guardian, and is managing his/her own financial affairs

   be approached for study participation for either themselves or their child?

   ☐ **Yes** ☑ **No**

5. Can or will wards of the state and/or children at risk of becoming wards of the state be enrolled (this includes foster children or any child that is in state custody)?

   ☐ **Yes** ☑ **No**

6. Can or will cognitively impaired adults (adults with impaired-decision making capacity) or adults who may lose the capacity to consent be enrolled?

   ☐ **Yes** ☑ **No**

7. Can or will prisoners be enrolled?

   ☐ **Yes** ☑ **No**

8. Can or will students and/or employees be enrolled in this research?

   ☐ **Yes** ☑ **No**

H. Adverse Event Monitoring

H.1 Definitions

Adverse Events: (Examples and not limited to)

- Hematoma
- Bleeding at needle puncture site
- Infection
- Hypotension

Serious Adverse Events:

- Death

- Methemoglobinemia
- Renal failure

H.2 Reporting Procedures

Study subjects will be monitored for the occurrence of adverse events defined as any clinically significant undesirable experience, from the day of enrollment until discharge. All adverse events will be recorded on an adverse event case report form and will include a description of all undesirable experiences, required interventions, subject's condition after the event, an estimate of the extent of injury and potential strategies to prevent future occurrences. The principal investigator will classify the relationship of the study protocol to the event. The principal investigator will report serious adverse events (death, life threatening, new, serious or permanent disability or adverse event culminating in hospitalization) according to IRB requirements. An adverse event is considered to be serious (SAE) if it results in any of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect

Non-serious adverse events will be summarized quarterly for the IRB. The adverse event case report form will include a description of all undesirable experiences or unanticipated benefits, required interventions, patient's condition after the event, an estimate of the extent of injury, and prevention strategies. The principal investigator will classify the relationship of the study protocol to the event as follows:

- Not related: An AE that is clearly and incontrovertibly due to extraneous causes (disease, environment, other drugs etc.)
- Unlikely related: An AE may be considered unlikely related if:
    - Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
    - Disease or other drugs provide plausible explanations
- Possibly related: An AE may be considered 'possibly related' if it meets at least 1 of the following criteria:
    - It follows a reasonable temporal sequence from administration of investigational product.
    - It may readily have been produced by the subject's clinical state or by environmental or toxic factors.
    - It follows a known response pattern to investigational product.
- Probably related: An AE that is considered to be related to investigational product with a high degree of certainty. An AE may be considered probably related if it meets all of the following criteria:
    - It follows a reasonable temporal sequence from administration of investigational product.

- It cannot be reasonably explained by the known characteristics of the subject's clinical state.
- It follows a known pattern of response to investigational product treatment.
- It reappears upon re-challenge

The severity of an adverse event in both groups is defined as a qualitative assessment of the degree of intensity of an adverse event as determined by the principal investigator as follows:

- Mild: Discomfort noticed, but no disruption to daily activity
- Moderate: Discomfort sufficient to reduce or affect normal daily activity
- Severe: Inability to work or perform normal daily activity

The principal investigator will follow-up on all serious events on a case-by-case basis. All adverse events will be summarized and reviewed on a quarterly basis

H.3 Reportable New Information

Reportable new information will be reported to the IRB per the Tufts Health Sciences IRB's Reportable New Information policy.

I. Statistical Considerations

I.1 Study Endpoints

1. Primary Endpoint: Prevention of progressive systemic de-oxygenation, with escalation to higher levels of oxygen and ventilatory support. Between-group differences in the average maximum disease severity score (Table 1) assessed through 28 days, through the following severity states: a) increased liter oxygen flow, through a high flow nasal cannula; b) non-invasive ventilation; c) intubation or institution of ECMO; or d) death.

2. **Secondary Endpoints:**

- Assessment based on an alternate 8-point Ordinal Outcome Scale, assigned daily from the data accrued, as above, through 28 days post-randomization or through discharge. Scale is as follows (a score of either 7 or 8 will be assigned at time of discharge):
  - 1 Death
  - 2 Hospitalized, requiring mechanical ventilation or ECMO
  - 3 Hospitalized, requiring non-invasive ventilation or high flow oxygen
  - 4 Hospitalized, requiring supplemental oxygen
  - 5 Hospitalized, not requiring supplemental oxygen - requiring ongoing medical care (COVID-19 related or otherwise)
  - 6 Hospitalized, not requiring supplemental oxygen - not requiring ongoing medical care (COVID-19 related or otherwise)
  - 7 Not hospitalized - limitation on activities and/or requiring home oxygen
  - 8 Not hospitalized, no limitations on activities

- Time to reaching maximal disease severity score
- Proportion of patients in each stage at maximum severity

- Dyspnea score, measured at baseline, 6±2 hrs after initiation and then daily until patient stops iNO or reaches 7 days
- PaO_2/FIO_2 or SaO_2/FIO_2 ratio, measured daily
- Length of hospital Stay (death assigned as worst case)
- Intubation, ECMO, or need to intubate with an "Do Not Resuscitate" order
- Mortality
- Levels of inflammatory mediators, including IL6, TNFα, fibrinogen, CRP, ferritin, D-dimer

I.2 Statistical Analysis

We plan to enroll 42 patients (28 iNO and 14 placebo). Data will be reported using means or medians, depending on patient distributions, and confidence intervals. The primary endpoint is the difference in average maximum disease severity between treatment groups through discharge, death or 28 days post-randomization.

Statistical Methods: Data analysis will be based on Intention-to-Treat. The primary endpoint will be compared using a two-sample t-test. If our data show significant departures from normality we will employ an appropriate non-parametric test. Secondary analyses will include comparisons between groups using the Chi-square or Fisher's exact test for categorical variables and two-sample t-test, repeated measures analysis, non-parametric statistical methods or time-to-event analyses as appropriate. Secondary analyses will include comparison of oxygen saturation and source as continuous variables. For this pilot study, no adjustments will be made for testing secondary hypotheses. P-values <0.05 will be considered significant.

Power considerations: Based on our experience with standard of care, we estimate that sample sizes of 28 and 14 in the study drug and control group, respectively, achieve 80% power to reject the null hypothesis of equal average maximum severity score when the mean is 3.36 in the standard of care group and 1.91 in the nitric oxide group with a standard deviation for both groups of 1.54 (effect size of 0.94) and with a significance level of 0.05 using a two-sided two-sample equal-variance t-test. The power calculation was performed using PASS 16.

I.3 Number of Subjects

We plan to enroll 42 patients (28 iNO and 14 placebo).

I.4 Data Management

Data collected will be stored and managed in a secure study database hosted through Tufts Medical Center. Access to the database will be managed by the clinical data manager. The study coordinator and the co-PIs of the study will enter data into the EDC via hospital-approved password-protected computers in locked offices in the hospital.

Stored Specimens:

a. Will be de-identified and labeled with study name, sample type, and purpose

b. Will be stored for 2 years in a -80^0C or colder freezer, at Tufts MC.

c. Only the Study Team will have access to specimen and data

I.5 Randomization

Will subjects be randomized?

☑ **Yes** ☐ **No**

If **Yes** respond to all of the following:

1. *Describe the randomization procedures, including the ratio of subjects randomized to each study arm:* Patients will be randomized 2:1 to blindly receive pulsed inhaled nitric oxide, in addition to standard of care, or placebo (inhaled nitrogen gas) in addition to standard of care in blocks of 6 subjects. A computerized block randomization program will be used.
2. *Describe the blinding procedures* or ☐N/A the study will not be blinded.

The research pharmacy will be responsible to randomize the patient and dispense the study drug in a blinded fashion. Please see section F2 for randomization process.

J. Drugs or Devices

1. Will the research involve drugs?

   ☑ **Yes** ☐ **No**

2. Will the research involve devices?

   ☐ **Yes** ☑ **No**

   If **Yes** to either, respond to all of the following:

   1. *Who on the research team, in addition to the Principal Investigator, will be accountable for drug(s):* The research coordinator, Abbey Haynes, as well as Dr Hill who is a Co-Investigator
   2. *Who will interface with the pharmacy?* Abbey Haynes and Dr. Diakos
   3. *If pre-printed orders will be created to obtain study drug(s) from the pharmacy, describe the procedures for reviewing and verifying the accuracy of the pre-printed orders prior to their being implemented:* *or* ☒N/A, there are no pre-printed orders
   4. *If computerized order sets are created and/or infusion devices need to be programmed to administer an investigational drug, indicate the mechanism to pre-review and verify their accuracy, including who will be involved in this process from the research team, pharmacy, and nursing:* or ☒N/A, there are no computerized orders sets and/or infusion devices.
   5. *The study drug, device, or procedure (including beneficial health care procedures) will be available to subjects after participation in the study:* ☐Yes ☒N/A
   6. *There are medications or other substances that should not be taken while participating in the study. A list of these are incorporated into the ICF a subject handout:* ☐Yes ☒N/A
   7. *Handouts or instructions sheets that will be given to subjects on how to administer study drug(s) or use study device(s) have been submitted to the IRB:* ☐Yes ☒N/A

K. Study Administration

K.1 Setting

1. *Describe the sites / locations where your research team will conduct the research:* Research will be conducted at in-hospital med-surg or critical care unit of Tufts Medical Center. Standard personal protective equipment will be mandated when it is necessary for any clinical trial personnel to enter the patient room.
2. *The research will take place at an international site, and the International Guidance and International Checklist were utilized:* ☐ Yes ☒ N/A

K.2 Registration

The study coordinator will ensure study subject eligibility and whether informed consent has been signed prior to any intervention.

K.3 Resources Available

Team:

- PI: Dr. Marvin Konstam is a board-certified cardiologist and advanced heart failure specialist who has extensive experience with the design and performance of clinical trials.

- In the absence of the PI, Dr. Hill will serve as acting PI.
- Co-Investigators: Dr. James E. Udelson, Dr. Iris Z. Jaffe and Dr. Navin K. Kapur are board certified cardiologists and will be responsible for data collection and analysis, screening, and consent processes.
- Co-Investigators: Dr. Nicholas Hill and Dr. Harrison Farber are board certified pulmonologist and critical care physicians and will be responsible for data collection and analysis, screening, and consent processes.
- Co- Investigator: Dr. John Adam Reich is a board certified anesthesiologist and critical care physician and will be responsible for data collection and analysis, screening, and consent processes.
- Co-Investigator: Leslie Lussier, MS, RRT, Director of Respiratory Therapy at Tufts Medical Center will oversee her team in instituting and maintaining therapy with iNO study drug, as well as managing administration of oxygen treatment. She will work closely with the research pharmacy to assure delivery of the correct agent to which the patient has been randomized.
- Co- Investigators: Dr. Nikolaos Diakos, Dr. Gaurav Gulati, and Dr. Mehak Dhande are cardiology fellows training at Tufts Medical Center and will be responsible for data collection and analysis, screening, and consent processes.
- Coordinator: Abbey Haynes is a clinical research coordinator and will be responsible for helping with data collection and analysis, screening, and consent processes

All study team members will be trained in the protocol and sign a delegation log acknowledging their responsibilities for the study.

K.4 IRB Review

1. ☒*Check to confirm that an appropriate IRB registered with the OHRP, will review and approve this study.*
2. ☒*Check to confirm that any amendments to the protocol or informed consent documents will be reviewed and approved by the IRB prior to use, unless required to eliminate an apparent immediate hazard to subjects.*

K.5 Multi-Site Research

Is this a multi-site study where Tufts is the sponsor, primary grant recipient, or coordinating site?:

☐ **Yes** ☑ **No**

K.6 Community-Based Participatory Research

Note: "Community-based Participatory Research" is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. Community-based Participatory Research begins with a research topic of importance to the community, has the aim of combining knowledge with action and achieving social change to improve health outcomes and eliminate health disparities.

Can or will this study involve community-based participatory research?

☐ **Yes** ☑ **No**

K.7 Sharing Results with Subjects

Will results (overall study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) be shared with subjects or others (e.g., the subject's primary care physician or the subject's treating physician)?

☐ **Yes** ☑ **No**

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