Clinical Trial Protocol: COVID-NONS-04

Study Title:	Multicenter, randomized, double-blinded, placebo-controlled, phase 3 clinical efficacy study evaluating nitric oxide nasal spray (NONS) as prevention for treatment of individuals at risk of exposure to COVID-19 infection
Study Number:	COVID-NONS-04 [NCT05109611]
Study Phase:	3
Product Name:	NONS (nitric oxide nasal spray)
Control Number:	Health Canada NOL c252244
EudraCT Number:	2021-004394-31
Indication:	Prevention of COVID-19 infection
Investigators:	Multicenter
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SYNOPSIS

Sponsor:
SaNOtize Research and Development Corporation

Name of Finished Product:
NONS (nitric oxide nasal spray)

Name of Active Ingredient:
NONS (nitric oxide nasal spray)

Study Title:
Multicenter, randomized, double-blinded, placebo-controlled, phase 3 clinical efficacy study evaluating nitric oxide nasal spray (NONS) as prevention for treatment of individuals at risk of exposure to COVID-19 infection

Study Number:
COVID-NONS-04

Study Centers:
This is a multicenter trial; however regional study centers will be established to facilitate study assessments and distribution of study treatment to participants

Study Phase: 3

Primary Objective(s):
To assess the efficacy of nitric oxide nasal spray (NONS) in the reduction of risk of coronavirus disease-19 (COVID-19) infection.

Secondary Objective(s):
To assess the efficacy of NONS in reduction of risk of the need for medical intervention resulting from COVID-19.

Assess tolerability of NONS in participants with COVID-19.

Study Design:
This is a multicenter, randomized, double blinded, placebo controlled, Phase 3 clinical efficacy study evaluating NONS in healthy adult volunteers as a prevention treatment for individuals at risk from COVID-19 infection. Participants aged 18 or over with no known history of SARS-CoV-2 infection will be enrolled into one of two cohorts of this study in a ratio of 1:1 (Treatment: Placebo Control). Participants with or without underlying medical conditions will be eligible to enroll.

Participants will self-administer a nasal spray three (3) times per day, morning, noon, and night containing either blinded study treatment or placebo (herein called study treatment). Nitric oxide nasal spray will be delivered from manual pump nasal spray bottle (containing 25 mL of solution) with each nasal spray dispensing approximately 130-150 µL of solution. Each treatment will require two sprays per nostril (1 spray per nostril, repeated once), or about 0.56 mL per treatment (4 sprays total per dose).

The primary endpoint is to assess the efficacy of NONS in the prevention of COVID-19 infection as determined by a positive COVID-19 test (antigen or reverse-transcriptase

polymerase chain reaction [RT-PCR]). Positive test results obtained within 48 hours of randomization will be considered infected at randomization and not counted as events.

The secondary endpoints are to determine the proportion for participants requiring hospitalization or emergency room (ER/emergency department [ED]) visits for COVID-19 or flu-like symptoms by Day 28 and assess the safety/tolerability of the use of NONS in participants for the prevention of COVID-19.

This study will be a multicenter trial, allowing remote and in-person on-site visits, including telephone contact, as applicable per respective country regulatory legislation. All data collection and monitoring to be performed remotely where possible. Screening and enrollment will take place through an online portal and through telehealth assessments, unless in a region that does not accept an eICF, in which case the consenting procedure will occur in-person on-site (screening and baseline procedures may also be performed in-person on-site). Participants will receive study treatment shipped directly to their homes unless in a region (European Union [EU]) or other location where the participant must receive the study product directly from the sites' staff/physician. Participants in the EU will have three in-person visits (screening visit, baseline visit [unless screening and baseline can occur on the same day] and a post-treatment visit). Participants will enter their study treatment dates and times and describe any symptoms in an online questionnaire (or paper diary cards if required).

Study Population:
The study population will consist of healthy volunteer males and females of any ethnic background aged at least 18 years of age with no prior history of a COVID-19 infection, or current SARS-CoV-2 infection.

Inclusion Criteria:

Each participant must meet the following criteria to be enrolled in this study.

1. At least aged 18 years old at the time of consent.
2. If female, be surgically sterile or post-menopausal (no menses for at least 12 months), or if of child-bearing potential, must be using an acceptable method of contraception such as a combination estrogen/progestin hormonal contraceptive (oral or injected) for at least 1 month prior to Day 1, or such items as an intrauterine device (IUD), intrauterine system (IUS), transdermal hormonal implant, vaginal hormonal ring, or two forms of the following: diaphragm, cervical cap, patch, condom, spermicide, or sponge. Total abstinence is permitted. If local regulations deviate from the previously listed contraception methods to prevent pregnancy, local requirements will apply. In addition, females of child-bearing potential must agree to continue to use their method of birth control for the duration of the study and 12 weeks following discharge from the study.
3. If male, be surgically sterile, or agree to use appropriate contraception (latex condom with spermicide) when engaging in sexual activity and agree to not donate sperm for the duration of the study and 12 weeks following discharge from the study.
4. Be in good health (ie, no acute illnesses or hospitalizations within 30 days of the study start, no planned procedures during study participation, and no newly diagnosed chronic illnesses that are not deemed stable by the participant's primary care

physician), in the opinion of the Investigator, based on medical history (ie, absence of any clinically relevant abnormality) during Screening.

5. Be able to understand and provide written, informed consent.
6. Access to a telephone, the internet, a device that reliability connects to the internet, and able to dial into Telehealth checkups and study related assessments is required; however, if access to the internet precludes involvement, then paper diaries may be completed and promptly entered into the data capture system at the study centers as part of regular follow-up visits.
7. Must be able to receive study product shipments directly to their home (ie, no Post Office Boxes), unless in a region (EU) or other location where the participant must receive the study product directly from the sites' staff/physician.

Exclusion Criteria:

Participants who meet any of the following criteria will be excluded from the study.

1. Participants with acute illnesses or hospitalizations within 30 days of the study start, and/or planned procedures during study participation, and/or newly diagnosed chronic illnesses that are not deemed stable by the participant's primary care physician), based on Investigator assessment of medical history during Screening.
2. Participants with any respiratory infection, flu-like symptoms, or unexplained fever or chills during the week prior to Screening.
3. Participants with any prior history of SARS-CoV-2 infection.
4. Participants who use intranasally dosed drugs, prescriptions or over-the-counter medications such as fluticasone within the last 7 days.
5. Participants who underwent a previous tracheostomy.
6. Participants who are receiving any form of oxygen therapy.
7. Females who are breastfeeding, pregnant, or attempting to become pregnant.
8. Participants who have any other condition that, in the opinion of the Investigator, would interfere with a participant's ability to adhere to the protocol (eg, participants whom are mentally or neurologically disabled and whom are considered not fit to their participation in the study), interfere with assessment of the investigational product, or compromise the safety of the participant or the quality of the data.

Planned Number of Participants: 13,000 to be randomized with a maximum of 11,480 participants initiating study treatment.

Test Product, Dose, and Mode of Administration: The test product is nitric oxide (NO) releasing solution delivered in the form of a nasal spray which will be self-administered three (3) times per day, morning, noon, and night for 28 days.

The comparator is a placebo of saline solution to be self-administered as the test product.

Duration of Treatment: Participants will be on study for 5 weeks (to include 1 week of screening/baseline, 4 weeks of treatment, 1 week of Follow-Up).

Efficacy Assessments: Participants will self-assess COVID-19-related symptoms, report if they have tested positive for COVID-19, and report if they need hospitalization or an ER visit. A positive COVID-19 test can be either by antigen or SARS-CoV-2 RT-PCR.

Safety Assessments: Adverse events (AEs) and discontinuations of treatment will be collected, and the number of participants that are hospitalized or visit the ED or ER for COVID-19/flu-like symptoms.

Study Endpoints: Primary Endpoint: Confirmed positive COVID-19 test (Both antigen and SARS-CoV-2 RT-PCR are acceptable) by Day 28.

Secondary Endpoint: Hospitalization or ER/ED visits for COVID-19/flu-like symptoms by Day 28.

Safety Endpoint: AEs and discontinuation of treatment.

Statistical Methods:

Determination of Sample Size: For an annualized infection rate of 3% in the placebo arm (daily incidence = 0.000075), and assuming a true hazard ratio of 0.15 implying protective effect of NONS equivalent to a vaccine efficacy of 85%, a sample size of 11,480 participants randomized 1:1 (NONS:Placebo) and available in the modified intent-to-treat (mITT) population will provide 90% power to demonstrate superiority of NONS to placebo in preventing COVID-19 infections reported through 28 days, based on Cox regression model and a 2-sided 0.05-level test. The expected number of events in the two arms is 2 and 10 for the NONS and placebo groups respectively. This further assumes 25% censoring during the 28-day period (1.05% loss per day).

Analysis Populations: Randomized participant, mITT, Per-Protocol, and the Safety population (all participants receiving any dose of treatment), the Per-Protocol population (received drug and had no major documented protocol violations).

Primary Efficacy Analysis: The primary efficacy objective is to demonstrate that NONS product reduces the risk of COVID-19 infection. The associated null hypothesis is that the hazard ratio (HR) of NONS relative to placebo is greater than 1, or H0: $HR \geq 1$, or equivalently in terms of a vaccine efficacy, H0: $VE \geq 0$ where vaccine efficacy is calculated as $VE = 100\% \times (1-HR)$. The HR will be estimated using a Cox proportional hazard regression model with a fixed effect only for treatment arm.

The estimand of the primary analysis is a treatment policy strategy and will consider intercurrent events as censored.

Secondary Endpoint Analysis: For the secondary objective of prevention of medical intervention for COVID-19, the secondary endpoint will be executed similarly to the primary efficacy analysis.

Safety Analysis: All safety analyses will be performed in the Safety Population. The primary analysis of safety will include counts and proportions of participants with AEs and SAEs summarized by Medical Dictionary of Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT) and cross-tabulated by severity, relationship to study treatment, treatment received, and overall. Exact Clopper-Pearson 95% confidence intervals for proportions may be constructed. All AEs and SAEs will be reported in a listing and events of interest will be itemized and listed separately.

Interim Analysis: Given the small number of expected events, there will be no interim analysis for efficacy. Participant enrollment may be closed before 11,480 subjects have entered the mITT population if 18 cases are accumulated, but this will be considered the final analysis, not an interim analysis.

Date of Original Approved Protocol: 12 May 2021

Document History Summary

Version / Date	Summary of Revisions
1.0 / 12 May 2021	Original Protocol
1.1 / 11 Aug 2021	Modification to a Multicenter trial from a decentralized trial. Expanded language to be globally relevant. Study product administration details added for clarity. Daily use population analysis added. EudraCT Number added. Administrative updates.
1.2 / 08 Dec 2021	Eligibility, updated to include an allowance of prior COVID-19 vaccination. Expanded option for paper-based questionnaire collection. Removal of antibody testing requirements. Relationship between Adverse Event and Study Drug, updated. Administrative updates.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ACE	Angiotensin converting enzyme
AE	Adverse event
$CCID_{50}$	Cell culture infectious dose 50%
CFR	Code of federal regulations
CI	Confidence interval
DMC/DSMC	Data monitoring committee/Data Safety and Monitoring Committee
eCRF	Electronic case report form
ePRO	Electronic patient-reported outcomes
CRO	Clinical Research Organization
ER/ED	Emergency room/emergency department
GCP	Good Clinical Practice
HC	Health Canada
HR	Hazard ratio
ICH	International Council for Harmonisation
ICF	Informed consent form
ICMJE	International Committee of Medical Journal Editors
IEC	Independent ethics committee
IOP	Internal Operating Procedures
IND	Investigational new drug
IRB	Institutional review board
IWRS	Interactive web response system
MedDRA	Medical dictionary for regulatory activities
MOP	Manual of procedures
mITT	modified intent-to-treat
NO	Nitric Oxide
NONS	Nitric oxide nasal spray
NORS	Nitric oxide releasing solution

PCR	Polymerase chain reaction
PP	per protocol
RT-PCR	Real-time polymerase chain reaction
SAE	Serious adverse event
SD	Standard deviation
SDV	Source data verification
SUSAR	Suspected unexpected serious adverse reaction
VE	Vaccine efficacy
WHO	World Health Organization

1 INTRODUCTION

1.1 Nitric Oxide as a Therapeutic Agent

Nitric oxide (NO) is a naturally occurring endogenously produced nano-molecule in most mammals, shown to possess a wide variety of biochemical characteristics that play a role in innate immunity, mammalian host defense against infection, modulation of wound healing, vasodilation, neurotransmission, and angiogenesis (Moncada, Palmer et al. 1991, Fang 1997) Nitric oxide properties such as antimicrobial, immunoregulatory, ciliary motility, and biofilm dispersal have utility in the treatment of a wide range of infections (Moncada, Palmer et al. 1991, Balijepalli, Comstock et al. 2015, Barraud, Kelso et al. 2015, Sheridan, Regev-Shoshani et al. 2016). Nitric oxide was approved as a selective pulmonary vasodilator used to treat "blue baby" syndrome over two decades ago (The Neonatal Inhaled Nitric Oxide Study Group 1997). Nitric oxide is widely recognized as a primary signaling molecule in biological systems and these effects are likely dose dependent (Schairer, Chouake et al. 2012). Despite these characteristics, NO has yet to be commercialized as an antimicrobial drug due to complex challenges with gaseous administration outside a hospital environment.

1.1.1 Nitric Oxide Viricidal Properties

Nitric Oxide is well-known to be an efficient broad-spectrum anti-infective agent. It has been reported to have antimicrobial activity against bacteria, yeast, fungi, and viruses both in vitro and in vivo animal studies (Rimmelzwaan, Baars et al. 1999, Ghaffari, Neil et al. 2005, Ghaffari, Miller et al. 2006, Regev-Shoshani, Ko et al. 2010, Regev-Shoshani, Church et al. 2013, Regev-Shoshani, Vimalanathan et al. 2013, Akaberi, Krambrich et al. 2020). Nitric oxide has been shown to have a direct effect on virions prior to infection and host cells already infected by viruses (Miller C 2008, Regev-Shoshani, Church et al. 2013, Regev-Shoshani, Vimalanathan et al. 2013).Nitric oxide, after nitrosylating cysteine moieties, causes conformational changes on surface glycoproteins. Nitric oxide also binds and block essential viral receptor sites on the host cell (Akerström, Gunalan et al. 2009). These conformational changes interfere with host cell fusion, preventing infection and release of virions from already infected host cells, and likely act similar to neuraminidase inhibitors (Akaberi, Krambrich et al. 2020).

1.1.2 Nitric Oxide Antiviral Properties

Within infected cells, NO interferes with messenger RNA (mRNA) transcription and replication by either inhibiting viral ribonucleotide (Croen 1993, Akarid, Sinet et al. 1995, Lin, Huang et al. 1997) or through inhibition of viral RNA synthesis (Sungnak, Huang et al. 2020). Other direct mechanisms could also account for the antiviral effects through viral DNA deamination or hydroxyl radical injury (Wink and Mitchell 1998). These effects were once attributed to peroxynitrite rather than NO, but this has since been disproven (Akerström, Gunalan et al. 2009). Nitric oxide was also shown to covalently bind and inhibit proteases and thus mitigate replication of the virus (Fang 1997). For this reason, a recently reported study concluded "given that nasal carriage is likely to be a key feature of transmission, drugs/vaccines administered intranasally could be highly effective in limiting spread of COVID-19 infection"(Sungnak, Huang et al. 2020).

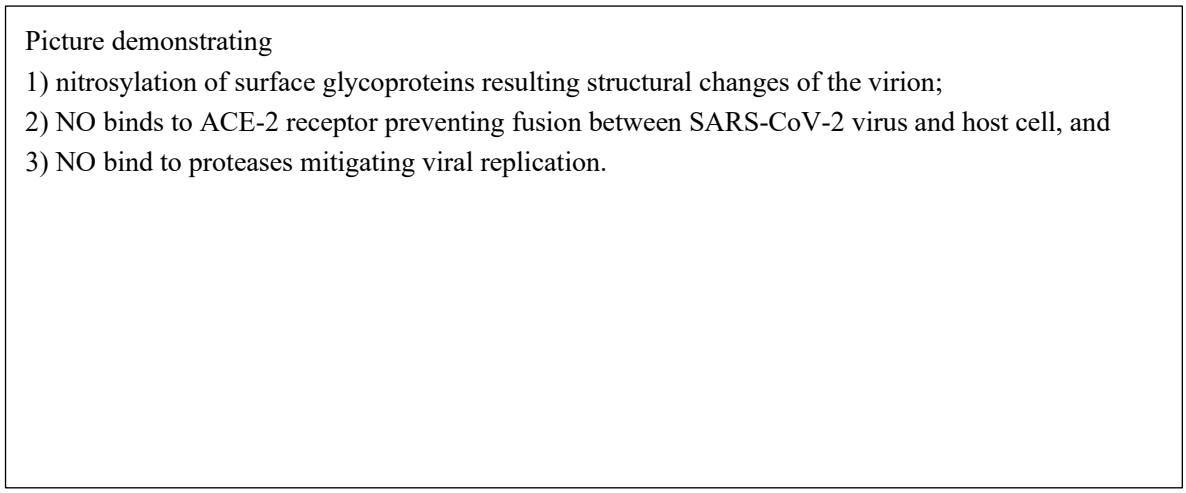
1.1.3 Angiotensin Converting Enzyme-2 Inhibition

The dual antiviral and angiotensin converting enzyme 2 (ACE-2) inhibition action of NO makes nitric oxide releasing solution (NORS) an attractive therapeutic to treat and prevent the spread of COVID-19 infection. NO has been shown to act as an ACE2 inhibitor which is highly expressed in the upper respiratory tract epithelial cells and the primary entry point of SARS-CoV-2 virus (Akerström, Gunalan et al. 2009). Infection of epithelial cells by the SARS-CoV-2 virus depends on ACE-2 as a receptor for cellular entry which is highly expressed in the nose (Li, Moore et al. 2003, Hoffmann, Kleine-Weber et al. 2020). The binding affinity of the viral Spike protein and ACE-2 was found to be a major determinant of SARS-CoV-2 replication rate and disease severity (Matsuyama, Nagata et al. 2010).

1.2 Nitric Oxide Mechanism of Action

Nitric oxide causes nitrosylation of surface glycoproteins resulting in structural changes of the virion particle. Nitric oxide binds to the ACE-2 receptor on epithelial tract cells preventing fusion between SARS-CoV-2 virus and the host cell, and NO bind to proteases mitigating viral replication (Figure 1).

Figure 1 Nitric Oxide Mechanisms of Action Against SARS-CoV-2



Nitric Oxide Mechanisms of Action Against SARS-CoV-2, 1) nitrosylation of surface glycoproteins resulting structural changes of the virion; 2) NO binds to ACE-2 receptor preventing fusion between SARS-CoV-2 virus and host cell, and 3) NO bind to proteases mitigating viral replication.
Abbreviations: ACE-2= angiotensin converting enzyme-2; NO= nitric oxide.

1.3 Nitric Oxide Releasing Solution (NORS)

SaNOtize has developed an innovative approach to deliver NO at doses that are lethal to microbes but safe to humans. SaNOtize's issued patent (US 9,730,956) describes the method and composition of NORS. This method combines the right formulation to produce an effective NO dose. NORS allows the delivery of varying amounts of NO, required to be an effective antimicrobial, for a wide range of indications. These characteristics enable NORS to

be a truly innovative platform technology for a variety of medical indications to eradicate multiple species of bacteria, fungi and viruses, including drug resistant organisms, as well as to reduce inflammation and increase wound healing. The development of NORS by SaNOtize was based on the antimicrobial data from pressurized NO gas studies. The effective antimicrobial NO dose was identified to be in the 120-200ppm per min range to eradicate a wide range of bacteria suspended in saline (See Investigator's Brochure Version 1.0). SaNOtize created a liquid, using two compounds abundantly used within the food industry, which contains an equivalent NO concertation as free gas to eradicate microbes.

The active drug used in this study is nitric oxide (NO). It is delivered within a saline-based liquid. The Sponsor proposes the use of an in situ activated, nitric oxide generating formulation that provides the same antimicrobial chemical characteristics in a solution as that of an exposure of 160 ppm NO gas to bacterial suspension in saline for 4 to 6 hours. This initial liquid with its specific chemical content is referred to by the Sponsor as Nitric Oxide Releasing Solution (NORS).

Nitric oxide nasal spray will be self-administered throughout the day. The intervention solution will be delivered in a manual pump nasal spray container (containing a dual chamber designed by the Sponsor) with 25 mL of solution. Each spray will dispense approximately 130-150µL of solution. Topical delivery of NONS will be performed with 4 nasal sprays at a time (two in each nostril) three (3) times daily, morning, noon, and night. The intervention solution will be delivered in a proprietary dual chambered (USP63/079,277) nasal spray container custom designed under cGMP for the Sponsor. The device is manufactured and formulated, filled/finished and packaged in by Nextar Chempharma Solutions (Nee Ziona, Israel). Each container holds 12.5 mL of liquid formulation and when activated by a manual pump, will mix equally the solutions in the head of the container to create NONS. When manually actuated, mixed NONS will be dispensed in a spray volume of approximately 130-150µL. The droplet size is greater than 5 µm to avoid droplets entering the lung field and maximize bulk application throughout the nasal cavity. Topical delivery of NONS will be performed with 4 nasal sprays at a time (two in each nostril) 3 times daily, morning, noon, and night.

1.4 Nitric Oxide Nasal Spray Effect on SARS-CoV-2 Clinical Isolates

In vitro tests conducted early in March 2020, by the Institute for Antiviral Research at Utah State University confirmed that NORS inactivated more than 99.9% of SARS-CoV-2 (below limit of detection) <0.7 cell culture infectious dose 50% ($CCID_{50}$) per 0.1 mL (>99.9%), within 2 minutes in laboratory tests using recent clinical isolates at titers of 10^4 PFU/ml (Figure 2). Testing was performed to determine if NORS samples inactivate viruses when exposed for a contact time of 2 minutes or 8 minutes.

After a 2-minute contact time, Dose B (similar NO release profile as NONS) reduced virus by from 3.9 to 1.8 log $CCID_{50}$ per 0.1 mL (>99%). After 8 minutes, Dose B reduced virus below the limit of detection, <0.7 $CCID_{50}$ per 0.1 mL (>99.9%). After the 2-minute and 8-minute contact time, Dose C (double NO release profile as NONS) reduced virus below the limit of detection of 0.7 $CCID_{50}$ per 0.1 mL (>99.9%).

Figure 2 Virucidal Effect of Nitric Oxide Spray

Graph depicts log 6 SARS-CoV-2 viral concentrations (log pu/mL) at baseline, followed by different doses (A, B, C) with viral growth measured at 30, 60 and 180 seconds.

No growth is demonstrated at all three timepoints for doses B & C.

No growth id demonstrated at 180 seconds for dose A, with log 2 growth shown at 60 seconds and log 3.5 growth at 30 seconds dose NORS/NONS.

Proprietary (Information)

Viricidal activity of two NORS doses against SARS-CoV-2 after two contact periods. $CCID_{50}$/well (log_{10}) was measured. Dotted line represents the limit of detection. Water was used as positive control. N=3. P<0.0001 as measured by one-way ANOVA with Dunnett's multiple comparison test.
Abbreviations: ANOVA= one-way analysis of variance; NORS= nitric oxide releasing solution.

1.5 Nitric Oxide Nasal Spray Effects in Humans

The Sponsor has conducted Phase 1 and Phase 2 clinical trials to help establish the safety of NORS as a localized topical antimicrobial and further demonstrate the lack of a systemic toxicity signal as measured by methemoglobin. These trials also provide environmental safety data demonstrating that nitrogen dioxide (NO_2) levels remain below detection limits and far below Occupational Safety & Health Administration (OSHA) guidelines.

1.5.1 Nitric Oxide Nasal Spray (NONS) for the Treatment of Mild COVID-19 Infection Trial

In 2020, the Sponsor conducted a double-blinded, placebo-controlled trial that involved 79 adults diagnosed with mild COVID-19 in the community (confirmed by polymerase chain reaction [PCR]). Participants were randomly assigned to treatment with NONS or placebo (saline solution) at day 1. Nasal and throat swabs were collected at presentation for baseline followed by collection at Days 2, 4 and 6 which were 1, 3 and 5 days after starting treatment, respectively. Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) was performed to determine SARS-CoV-2 RNA. Nitric oxide nasal spray, started on or before day 5 of symptom onset, was independently associated with a significantly accelerated decrease in SARS-CoV-2 RNA concentration of -1.21 log_{10} copies/ml on both Days 2 and 4, as compared to placebo. Therefore, the mean SARS-CoV-2 RNA concentration was lower for NONS, as compared to placebo, by a factor of 16 at both Days 2 and 4. Day 6 equated to at least 10 days since symptom onset and significant reduction of SARS-CoV-2 with or

without treatment was expected. Nevertheless, the SARS-CoV-2 RNA concentration was still lower on NONS at Day 6. The primary endpoint was a reduction of viral load from Day 1 through Day 6 (measured as area under the curve). Analysis revealed a mean difference of -5.22 $\log_{10}$ copies/ml between NONS and placebo. This difference was shown to be significant with a P value of 0.0096. The average viral log reduction in the first 24 hours, in treatment arm was -1.362 which correlates to about 95%, while the average viral log reduction in the control (saline) arm was -0.15 which correlates to about 30% In addition, the majority of patients in this study were positive for SARS-CoV-2 variants of concern (VOC202012/01) also commonly known as the UK variant (European Medicines Agency 2020).

1.5.2 Nitric Oxide Nasal Spray COVID-19 Prevention Trial

In 2020, the Sponsor closed enrollment of a Health Canada (HC) trial (NCT04337918) to evaluate the safety and efficacy of NORS to prevent infection from SARS-CoV-2. In total, 143 participants were enrolled, and 103 participants received the NORS interventions in the form of NONS. Over 7,000 doses of NONS were administered in this trial and there were no reports of serious adverse events (SAEs) or significant adverse reactions (ARs) related to NONS specifically. Some participants reported adverse events (AEs) of mild nasal irritation which quickly resolved. None of the participants in the intervention arm (N=103) tested positive for COVID-19 during the study. However, no participants in the control arm (N=40) tested positive either so efficacy was not shown.

1.5.3 Nitric Oxide Nasal Spray Background

The NORS formulation is manipulated to achieve a safe dose that is microbicidal within minutes to viral and bacterial pathogens. Specific to pathogens related to sinusitis, it is called Nitric Oxide Nasopharyngeal Irrigation (NONI) or Nitric Oxide Gargle (NOG) or Nitric Oxide Nasal Spray (NONS). Based on scientific data (see Investigator Brochure Version 1,0 for details) the NO releasing capability of these products, should act as a nitrosative viricidal agent, may have beneficial restorative mucociliary and anti-inflammatory effects in participants with Flu/COVID-19.

Prevention is essential in controlling the spread of COVID-19. The Sponsor's nitric oxide nasal spray (NONS) would provide a new daily preventative measure as an easy-to-use prophylaxis. Nitric oxide nasal spray aims to initially reduce the pathogen load in the nasal passages where viruses first enter and reside and then blocks viral entry before they can penetrate the cells lining the nose.

Nitric oxide nasal spray will be self-administered throughout the day. The intervention solution will be delivered in a manual pump nasal spray container with 25 mL of solution. Each spray will dispense approximately 130-150µL of solution. Topical delivery of NONS will be performed after the individual blows their nose, with 4 nasal sprays at a time (two in each nostril) 3 times per day.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective and Endpoint

Objective	Endpoint
To assess the efficacy of NONS in the reduction of risk of COVID-19 infection	Confirmed positive COVID-19 test (Both antigen and SARS-CoV-2 RT-PCR are acceptable) by Day 28. Positive test results based on sample collection within 48 hours of randomization will be considered infected at randomization and not counted as events.

Abbreviations: NONS=nitric oxide nasal spray; RT-PCR=real-time polymerase chain reaction.

2.2 Secondary Objectives and Endpoints

Objective	Endpoint
To assess the efficacy of NONS in prevention of severe COVID-19	Hospitalization or ER/ED visits for COVID-19/flu-like symptoms by Day 28
Assess tolerability of NONS in participants with all participants including those with COVID-19	AEs and discontinuation of treatment

Abbreviations: AE=adverse events; ER/ED=emergency room, emergency department NONS=nitric oxide nasal spray.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a multicenter, randomized, double-blinded, placebo controlled, Phase 3 clinical efficacy study evaluating NONS in healthy adult volunteers as a prevention treatment for individuals at risk from COVID-19 infection.

Up to 13,000 adult participants aged 18 or over with no known history of SARS-CoV-2 infection will be randomized into one of two cohorts of this study in a ratio of 1:1 (Treatment: Placebo Control). Participants with or without underlying medical conditions will be eligible to enroll. The NONS formulation proposed for use in this COVID-19 clinical study will be self-administered at a maximum of 3 times per day, morning, noon, and night for 28 days. Participants will be on study for 4 weeks of treatment (to include 5 days of Screening, 1 week of Follow-Up, participants will be followed for a total of approximately 5 weeks) as a viricidal Investigational Medical Product.

Participants will self-administer a nasal spray containing either blinded study treatment or placebo (herein called study treatment). Nitric oxide nasal spray (NONS) will be delivered from manual pump nasal spray container with 25 mL of solution with each nasal spray dispensing approximately 130-150 µL of solution. After the subject blows their nose, each treatment will require two sprays per nostril, or about 0.5 mL per treatment.

The primary endpoint is to assess the efficacy of NONS in the prevention of COVID-19 infection as determined by a positive COVID-19 test (antigen or reverse-transcriptase polymerase chain reaction [RT-PCR]),

The secondary endpoints are to determine the proportion for participants requiring hospitalization or emergency room (ER/emergency department [ED]) visits for COVID-19 or flu-like symptoms by Day 28 and assess the safety and tolerability of use of NONS in participants with COVID-19.

This study will be a multicenter trial, allowing remote and in-person on-site visits, including telephone contact, as applicable per respective country regulatory legislation. All data collection and monitoring to be primarily performed remotely (Clinical Trials Transformation Initiative 2018). Screening and enrollment will take place through an online portal and through telehealth assessments, unless in a region that does not accept an eICF, in which case the consenting procedure will occur in-person on-site (screening and baseline procedures may also be performed in-person on-site). Participants will receive study treatment shipped directly to their homes as described in Section 6.5., unless in a region (EU) where the participant must receive the study product directly from the sites' staff/physician. Participants in the EU will have three in-person visits (screening visit, baseline visit [unless screening and baseline can occur on the same day] and a post-treatment visit). Participants will enter their study treatment dates and times and describe any symptoms in an online questionnaire (Table 4), or paper diary if necessary. Study sites will maintain contact with all enrolled participants and regularly collect paper diaries to ensure timely data entry.

3.2 Rationale for Study Design and Control Group

3.2.1 Rationale for Use of Nitric Oxide for COVID-19 Therapy

SARS-CoV-2 is a novel, human-infecting coronavirus, termed COVID-19. COVID-19 is highly contagious, with a high virulence, and on March 11, 2020 it was declared by the World Health Organization a global pandemic. A study treating individuals with a viricidal agent like NORS could be advantageous for many reasons. This treatment could be used either for infection prevention or at the time of diagnosis when the virus is the most susceptible because it is still in the nose or in upper airways. This approach could prevent the population in viral outbreak areas from getting infected, mitigate the progression of the viral infection and spread of the disease from those who are infected already to others. Obtaining an early diagnosis when the patient has mild to moderate disease and treating them while spontaneously breathing, could translate in the reduction of morbidity and mortality. Thus, it could lower the burden on already limited healthcare resources.

3.2.2 Rationale for use of a Nitric Oxide Releasing Solution Spray (NONS) Formulation

It is hypothesized that a NORS formulation, delivered prophylactically or at early stages of COVID-19 exposure, will be well tolerated, and may have other beneficial secondary outcomes. In addition, due to nitric oxide's broad spectrum, non-specific antimicrobial (specifically viricidal) characteristics and its fundamental basic biochemical action, microbial drug resistance may even be avoided. This is especially important with the emergence of variants that reduce the effectiveness of vaccines.

Nitric oxide releasing solution will be delivered in a nasal spray formulation, referred to herein as nitric oxide nasal spray (NONS). Given the severity and persistence of the complications associated with COVID-19 infection, the Sponsor hypothesizes that nitric oxide delivered via NONS may prevent COVID-19 infection.

While there have been only minimal reported side-effects from the topical application of gaseous nitric oxide, there have been reported adverse side effects of nitric oxide releasing compounds. Specifically, the adverse effects associated with topical nitrate/nitrite application include skin rash, burning sensation, sensitivity and staining of the skin and are mostly associated with the carrier or the acid required for NO donor release (Weller, Price et al. 1997).

The dose for the NONS is based on preliminary in-vitro dosing studies, using H1N1 and H3N2 and SARS-CoV-2 viruses, and based on the highest tolerated dose in volunteers in previous clinical trials conducted by the Sponsor.

3.2.3 Rationale for Multicenter Trial Design

The study will be conducted in a blinded fashion in conjunction with a placebo control to remove evaluation biased by the expected effects of the study treatment.

If allowed by applicable legislation in the respective country, all data collection and monitoring will be remote through telehealth assessments and phone calls without performing on-person visits. This allows for broader geographic reach and rapid response, for easy access to areas that present with a higher prevalence of the COVID-19 infection.

In countries where only remote visits are applicable, all participant interactions with the Investigator will occur through telehealth assessments in the participant's home (see Section 6.6.2 and Section 6.6.3 for more details on telehealth assessments). The participant will receive study treatment directly to their home, unless in a region (EU) or other location where the participant must receive the study product directly from the sites' staff/physician. All study treatments will be self-administered. A completely remote option trial design allows for potentially faster and more diverse participant recruitment, improved participant retention, which may reduce missing data, and provides the participant with greater control, convenience, and comfort performing treatments in the home. The administration of treatments in the home may also be more representative of real-world administration and use of the study treatment post-approval.

3.3 Study Duration and Dates

Actual overall study duration or participant recruitment period may vary. The proposed study start is Q4 2021. A participant's time on the study is expected to last 5 weeks from enrollment. Subject participation will consist of 5 days of Screening/Baseline, and 28 days study treatment, followed by 7 days of Follow-Up for safety assessments. Participants should complete the study on Day 35.

For participants that test positive for COVID-19, Health Canada (HC)/FDA, or other applicable relevant local/national regulatory health authorities should be notified as applicable to the regional area. Participants that test positive for COVID-19 will be encouraged to complete early termination assessments and record their symptoms using the COVID-19 symptom list.

3.3.1 End of Study Definition

A participant is considered to have completed the study if they have completed all phases of the study including Follow-up (Day 35), or they/their emergency contact completes the early termination assessment questionnaire after the last treatment. The end of the study is defined as the date of the last assessment of the last participant.

4 STUDY POPULATION SELECTION

4.1 Study Population

The study population will consist of healthy volunteer males and females of any ethnic background aged at least 18 years of age with no prior history of a COVID-19 infection, or current SARS-CoV-2 infection.

If allowed by local regulations, participants will be recruited through public announcements and social media as approved by the ethics review board(s). Ideally, cooperation with Public Health Officials will aid in identifying "hotspots" where focused advertising for recruitment will be targeted. A study website may be set up to support initial interest and screening efforts. Additional efforts to support study visibility and broaden recruitment areas to more remote locations, will be assessed throughout the course of the study such as television or radio advertising. Local/national regulations regarding recruitment of participants for clinical research will be applied in each country.

Participants may be recruited in multiple countries, starting in Sri Lanka, using a multicenter method, it is estimated that up to 13,000 participants will be randomized with an anticipated 11,480 participants initiating study treatment. A screened participant is defined as someone who has expressed interest in the study but has not completed the consent and randomization process.

4.2 Inclusion Criteria

Each participant must meet the following criteria to be enrolled in this study.

1. At least aged 18 years old at the time of consent.
2. If female, be surgically sterile or post-menopausal (no menses for at least 12 months), or if of child-bearing potential, must be using an acceptable method of contraception such as a combination estrogen/progestin hormonal contraceptive (oral or injected) for at least 1 month prior to Day 1, or such items as an intrauterine device (IUD), intrauterine system (IUS), transdermal hormonal implant, vaginal hormonal ring, or two forms of the following: diaphragm, cervical cap, patch, condom, spermicide, or sponge. Total abstinence is permitted. If local regulations deviate from the previously listed contraception methods to prevent pregnancy, local requirements will apply. In addition, females of child-bearing potential must agree to continue to use their method of birth control for the duration of the study and 12 weeks following discharge from the study.
3. If male, be surgically sterile, or agree to use appropriate contraception (latex condom with spermicide) when engaging in sexual activity and agree to not donate sperm for the duration of the study and 12 weeks following discharge from the study.
4. Be in good health (ie, no acute illnesses or hospitalizations within 30 days of the study start, no planned procedures during study participation, and no newly diagnosed chronic illnesses that are not deemed stable by the participant's primary care physician), in the opinion of the Investigator, based on medical history (ie, absence of any clinically relevant abnormality) during Screening.

5. Be able to understand and provide written, informed consent.
6. Access to a telephone, the internet, a device that reliability connects to the internet, and able to dial into Telehealth checkups and study related assessments. If access to the internet precludes involvement, then paper diaries may be completed and promptly entered into the data capture system at the study centers as part of the regular follow-up visits.
7. Must be able to receive study product shipments directly to their home (ie, no Post Office Boxes), unless in a region (EU) or other location where the participant must receive the study product directly from the sites' staff/physician.

4.3 Exclusion Criteria

Participants who meet any of the following criteria will be excluded from the study.

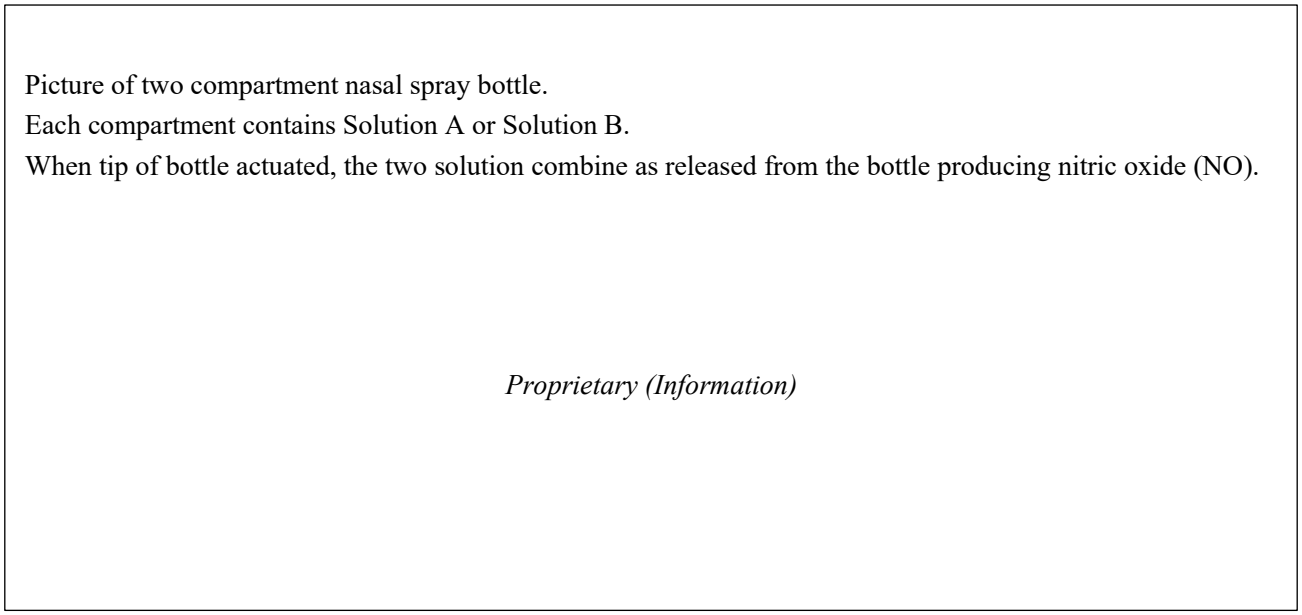
1. Participants with acute illnesses or hospitalizations within 30 days of the study start, and/or planned procedures during study participation, and/or newly diagnosed chronic illnesses that are not deemed stable by the participant's primary care physician), based on Investigator assessment of medical history during Screening.
2. Participants with any respiratory infection, flu-like symptoms, or unexplained fever or chills during the week prior to Screening (see Section 6.6.2).
3. Participants with any prior history of SARS-CoV-2 infection.
4. Participants who use intranasally dosed drugs, prescriptions or over-the-counter medications such as fluticasone within the last 7 days.
5. Participants who underwent a previous tracheostomy.
6. Participants who are receiving any form of oxygen therapy.
7. Females who are breastfeeding, pregnant, or attempting to become pregnant.
8. Participants who have any other condition that, in the opinion of the Investigator, would interfere with a participant's ability to adhere to the protocol (eg, participants whom are mentally or neurologically disabled and whom are considered not fit to their participation in the study), interfere with assessment of the investigational product, or compromise the safety of the participant or the quality of the data.

5 STUDY TREATMENT

5.1 Description of Treatment

The Sponsor designed a dual chamber nasal spray bottle for NONS administration. Components are mixed from two chambers to create the final NO-producing formulation. The liquid contains NO at 0.11 ppm*hour, which acts as a viricidal agent (Figure 3). Instructions for storing, preparing, and administering the study treatment will be provided to participants.

Figure 3 Nitric Oxide Nasal Spray



Abbreviations: hr= hour; NO= nitric oxide, ppm= parts per million

5.2 Treatments Administered

Nitric oxide nasal spray or placebo will be self-administered by the participant three (3) times throughout the day, at approximately in the morning, noon, and night (or about every 8 hours) for 28 days. The intervention solution will be delivered in a manual nasal spray pump bottle container with 25 mL of solution. Each spray will dispense approximately 130-150 µL of solution and each treatment or dose requires 4 total sprays (delivered as 1 spray in each nostril, and then repeated as 1 spray in each nostril). Total exposure to study intervention will be a maximum of 84 treatments totaling 50 mL (three 25 mL spray bottles will be dispensed to ensure enough solution volume is available to participants, including coverage for priming the bottles, malfunctioning bottles and/or misplaced/lost bottles). All participants will be monitored for 7 days post-last treatment dose (to Day 35) as shown in Figure 4. Refer also to the Dosing Instructions for Participants.

Figure 4 Study Schema

Schema available upon request

IP: in-person on-site visit

5.3 Selection and Timing of Dose for Each Participant

After screening has been completed, participants will be randomized by an interactive web response system (IWRS) into one of two groups, one group of 6,500 for the NONS treatment and one group of 6,500 for placebo treatments. Following randomization, study treatment will be dispensed during the in-person on-site visit (as required by local/national regulations) or shipped directly to participants for immediate use and confirmation on the appropriate eCRF. Participants will be instructed to treat themselves 3 times daily, morning, noon, and night (or approximately every 8 hours) with the nasal spray for 28 consecutive days.

5.4 Method of Assigning Participants to Treatment Groups

Participants will be randomized 1:1 to receive either NONS or placebo. Randomization will be in a double-blinded manner using an IWRS with pre-generated randomization lists. When participants performing remote visits (only) are confirmed as eligible, the IWRS system will provide the participant's contact information and randomized assignment to the regional drug distribution center who will compile the kit with the appropriate 3 bottles of test articles (NONS or placebo) and ship them directly to the participant.

Participants may only enroll on the study once. During eligibility screening, data will be collected verifying identity (date of birth and partial postcode) to prevent participants from attempting to enroll the study again.

Randomization will be stratified based on age and COVID-19 risk factors. There will be three strata:

1. ≥ 65 years of age
2. < 65 years of age and at increased risk
3. < 65 years of age and not at increased risk

Participants will be considered 'at increased risk' if they report having at least one of the following risk factors during Screening:

- Cancer
- Chronic lung disease (eg: emphysema and chronic bronchitis, idiopathic pulmonary fibrosis and cystic fibrosis) or moderate to severe asthma
- Significant cardiac disease (eg: heart failure, coronary artery disease, congenital heart disease, cardiomyopathies, and pulmonary hypertension)
- Down Syndrome
- Immunocompromised following immunosuppressive therapies (e.g. post-transplant procedures, chemotherapies, treatments with biologics/biosimilars)
- COPD (chronic obstructive pulmonary disease)
- Obesity (body mass index [BMI] ≥ 30 kg/m^2)
- Diabetes (Type 1, Type 2 or gestational)
- Liver disease
- HIV infection

5.5 Blinding

Following confirmation of eligibility and baseline data collection, participants will be enrolled and randomized.

If an in-person on-site visit is performed, blinded kits will be delivered to, or prepared at, the sites. A subject number will be assigned to each randomized participant. Site staff/physician will then dispense kit labeled with the authorization number generated during randomization to the individual participant.

For participants performing remote visits, each authorization number/code will be associated with a specific treatment assignment, within stratum, from a pre-generated list. The authorization number/code will be provided to the regional study drug distribution center(s) who will select the appropriate treatment assignment, compile a blinded kit, and distribute directly to the participant. In addition to the necessary staff at the study drug distribution center, unblinded staff at the study contract research organization (CRO) will be the only study personnel with access to unblinded treatment assignments.

If the Investigator, or any medical caregiver, determines that unblinding is warranted to manage a medical condition, AE, or other purpose, they should follow the specified contact

procedure in the study Safety Monitoring Plan – contact information will be available to study participants to provide to physicians or other medical caregivers who may be providing care to the subject. Safety must always be the first consideration in making a determination to unmask. The requestor should believe that knowledge of the participant's randomized study treatment would influence the participant's care or their treatment decisions. Upon approval by the Sponsor, or in the case of urgent medical need, the unblinding information will be provided with notification to the Sponsor and documentation of the unblinding event in the study file.

5.6 Concomitant Therapy

If possible, participants should be maintained on stable medications and dosing throughout the entire study period, as medically feasible. Concomitant medications (including COVID-19 vaccination) other than naturopathic products will be recorded for the duration of the study.

The use of standard delivery nasal sprays including over-the-counter medications and generic formulations such as fluticasone are prohibited whilst the participant is on the study. This includes all forms of nasal therapy (e.g. nasal lavage of any kind, steam inhalation of any kind, or any other homeopathic nasal treatments) that could interfere with study outcomes.

Participants requiring an initiation of new medications or treatment for COVID-19 at any point will contact the appropriate study staff personnel, i.e., investigator/study coordinator, at the regional site/office.

5.7 Lifestyle Considerations

5.7.1 Prior Therapy

The Investigator or qualified designee will review prior medication use with the participant and record the use of any prescription or non-prescription drugs taken by the participant within 14 days before Day 1.

5.7.2 Fluid and Food Intake Considerations

Participants should maintain their usual diet.

5.7.3 Patient Activity Restrictions

Participants should maintain their usual levels of activity.

5.8 Screen Failures

For participants who are Screen failures and do not receive any study treatment, their demographics and the reason for Screening failure will be recorded. The same information will be recorded for participants that pass Screening but subsequently are not randomized and do not receive any study treatment.

5.9 Treatment Compliance

As participants are self-administering the study treatment at home, compliance with study treatment will be reviewed at each contact with the participant (including telehealth assessments) and the eCRF treatment diaries or paper diaries. Participants will complete a treatment diary at home for the dosing days. Any safety issues noted on the electronic treatment diary or paper diary will be recorded in the eCRF and reviewed by the regional Investigator. Participants that do not confirm use of the treatments on the appropriate eCRF may be terminated early at the discretion of the investigator.

If a participant develops clinical symptoms of COVID-19, they should document these in a COVID-19 symptoms diary (Appendix 2).

Refer to the Manual of Procedures (MOP), Internal Operating Procedures (IOP) or supply management plan for study treatment accountability and returns.

6 STUDY PROCEDURES

Details of the timing of study procedures are provided in Table 4.

6.1 Informed Consent

Potential participants will be offered participation in the study. The informed consent will be obtained as per applicable legislation in the respective country. Potential participants will be invited to the site where they will receive the Informed Consent Form document and the investigator will explain the study and answer any questions related to the study. Potential participant will be given as much time as needed to decide if they are willing to participate.

In countries where Informed Consent Form is allowed to be obtained remotely as per applicable legislation, the informed consent document will then be sent electronically to the potential participant, whom will be given as much time as possible to review and ask questions by telephone or virtual meeting as institutional standards allow with research ethics board approval. Once the participant has signed the form, he or she will receive an electronic copy. The Investigator can then access the signed consent in a secure manner and print it or transfer the file electronically (Table 4). Electronic signature collection software will comply with 21 CRF part 11 (Part 11).

6.1.1 Emergency Contact Informed Consent

Participants will be asked to designate an emergency contact person and alternate contact telephone number during consent. The emergency contact will be asked to provide written, informed consent to perform these duties as per local regulations.

The emergency contact will be contacted in the event the participant is hospitalized and is unable to complete their daily electronic diary. Emergency contacts are encouraged to submit study data on behalf of the participant (as applicable).

6.2 Screening Assessment

Screening will be performed on-site unless specific geographic jurisdiction allows remote screening procedures. Remotely consented participants will be referred to a regional study center for a telehealth evaluation by a principal or sub-Investigator who is a medical provider qualified to order testing and prescribe medication for a specific geographic jurisdiction. Screening procedures will be performed as described in the Schedule of Events (Table 4). Participants will be enrolled on the study if determined eligible and assigned a randomization number.

6.3 Demographics, Medical History, and Physical Examination

Demographic information (month and year of birth, sex, ethnicity, and race) will be collected as described in the Schedule of Events (Table 4).

Concomitant medication information will be collected as described in the Schedule of Events (Table 4).

The investigator (qualified medical provider if remote screening is allowed) will complete a pertinent medical history and current concomitant medications (refer to the MOP/IOP) and physical examination (remotely if applicable), including an assessment of any flu-like symptoms (see Section 6.6.2) as described in the Schedule of Events (Table 4).

All medical history findings that have been present/active within the 3 years prior to enrollment will be recorded regardless of clinical relevance or presence at study start. Medical history findings that have not been present/active within the 3 years prior to enrollment will be recorded if deemed clinically relevant by the Investigator to the conduct of the study. The medical history should include any history of allergic reactions to drugs.

6.4 Clinical Laboratory Assessments

6.4.1 Urine Pregnancy Test

A urine pregnancy test will be required from female participants of childbearing potential as described in the Schedule of Events (Table 4). Self-reported urine pregnancy test will be used if remote screening is applicable.

A negative urine pregnancy test is required prior to enrolling the participant in the study. The negative pregnancy test will be confirmed during Screening and prior to dosing as described in the Manual of Procedures (MOP) or Internal Operating Procedures (IOP).

6.5 Dispensing Study Drug

Study drug will be dispensed during the baseline on-site visit per applicable local/national legislation. Remotely enrolled participants will be sent overnight, a direct to participant temperature-controlled and monitored package which will contain a 28-day supply of study treatment and instructional materials as described in the MOP/IOP. The instructional materials will consist of study treatment administration, contact information for reporting safety concerns, and what to do if they are diagnosed with COVID-19.

Three nasal spray bottles will be provided. Participants are to self-administer study treatment at 3 times (doses) per day for 28 consecutive days. Participants will record in their diaries the dosing date and time when they administer each study treatment.

There are no preparation steps for the nasal spray bottle (after it is primed). Each dose will be 4 sprays in total. The technique for spraying will be to first blow the nose to clear mucous debris. The tip of the bottle should be barely placed in the opening of the nostril. Begin with a single spray to the lateral inside of the nose of each nostril (1 spray in each nostril). Then proceed with a single spray directly into the nose of each nostril (1 spray in each nostril, for a total of 4 sprays per dose) while breathing in (note that this maneuver may cause a slight astringent sensation).

The participants will be instructed on nasal spray technique and instructed the day before dosing to ensure consistency with understanding and technique (an online video link will be provided).

6.6 Efficacy Assessments

Participants will self-assess COVID-19-related symptoms (Section 6.6.2), report if they have tested positive for COVID-19, and report if they need hospitalization or an ER/ED visit as described in the Schedule of Events (Table 4). A positive COVID-19 test can be either by antigen or SARS-CoV-2 RT-PCR.

6.6.1 Daily Treatment Diary and Questionnaire (eCRF)

The questionnaire and electronic treatment diary will record the compliance of treatments administered or missed. It will also ask if treatments were tolerated and report any side effects or other comments. There is also a discomfort/pain scale if needed. Refer to Section 8.7 for action to be taken in the event of the spray bottle malfunctioning. Paper diaries with comparable information and instructions will be available as backup for all participants or as primary collection instruments for participants without sufficient access to devices or internet connectivity.

All participants will be asked to fill out an online (or paper if required) daily treatment questionnaire and COVID-19 symptoms diary as described in the Schedule of Events (Table 4). The complete language and content of all of the questions is described in the Manual of Procedures.

Participants must have access to the internet and a device that reliability connects to the internet and is able to dial into telehealth assessment. In the event of an inability to access the electronic PRO, the process described in the MOP/IOP will be followed for completion of paper records.

If a participant develops clinical symptoms of COVID-19 (see Section 6.6.4), they/their emergency contact should make every effort to document these in a COVID-19 symptoms list (Appendix 2).

Participants who enroll in the study could be asymptomatic for COVID-19 at time of randomization; in which case, if the participant tests positive for COVID-19 within < 2 days (48hrs) of the baseline visit they will be considered unevaluable for the study endpoint and will be discontinued.

6.6.2 In-Person On-Site/Telephone Contact

Study staff for sites requiring on-site visits will contact participants via telephone on Day 7 and 28 (all others performing remote monitoring will contact participants on Day 7, 28, and 35) as described in the Schedule of Events (Table 4) and confirm participants' current health status including presence of any COVID-19 symptoms (Appendix 2; Appendix 3), in addition to any ER or hospital visits that might have occurred as a result of COVID-19. Participants will also be asked about any challenges with administration or any AEs they may have experienced.

Participants who are hospitalized for any reason and are unable to attend their on-site visit / telehealth assessment or complete their daily diary should have their emergency contact notify study staff as soon as possible. Participants that are hospitalized for COVID-19 only are considered treatment failures. Participants that are hospitalized for any other reason may stay on the study at the discretion of the Investigator.

Follow-up visits (end of study and early termination assessments) will be performed preferably on-site (in countries where remote only conduct is not allowed), but if the participant is hospitalized, in quarantine or unable to visit the site in-person for any reasonable cause as per investigators' judgement, these visits may be performed remotely and will not be considered a protocol deviation. Follow-up of all adverse events must be completed as described in the protocol Section 7.

6.6.3 Remote Video Conferencing

All study staff who will be conducting remote telehealth assessments should be trained on how to conduct real-time video conferencing assessments. Procedures will be put in place to maintain participant privacy as in an in-person visit. Both the Investigator and the participant should confirm their identities to one another before engaging in a real-time video conference assessment (National Insttitue of Standard and Technology (NIST) Digital Identity Guidelines 2017, Royal College of Physicians and Surgeons of Canada 2020, Food and Drug Administration 2021).

6.6.4 COVID-19 Symptoms List

Health Canada COVID-19 symptoms list: https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/symptoms.html

Center for Disease Control (CDC) COVID-19 symptoms list: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

6.6.5 Early Termination Assessment

Participants that discontinue the study early due to COVID-19 infection or are hospitalized for any reason are encouraged to attend the early termination assessment to the best of their ability. If the participant is unable to complete the treatment diary or attend the early termination assessment, the designated emergency contact is encouraged to submit this information on behalf of the participant to the best of their ability.

Protocol deviations to be recorded for this study include visit/phone/telehealth procedure deviations and noncompliance; PRO noncompliance; study medication dosing noncompliance; and dispensing deviations. A special emphasis will be placed on dosing errors, lack of completion of the required daily PRO reporting, and the incomplete return of study medication at the conclusion of the study.

Study medication noncompliance is defined as less than 80% of expected doses administered while on possible study treatment. PRO noncompliance is defined as less than 70% of possible reporting days of data recorded, eg. before a hospitalization, or an early termination.

7 ADVERSE EVENTS ASSESSMENTS

7.1 Adverse Event Definition

As per Good clinical Practice (GCP) guidelines, an AE is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

AEs will include (1) any new medical condition (sign/symptom/disease) that occurs during the AE reporting period, or (2) any preexisting condition that worsens in severity or frequency or changes in character during the AE reporting period.

An SAE is defined as an AE that, in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is a life-threatening AE
  - A life-threatening AE is defined as an AE that in the view of either the Investigator or Sponsor, places the patient or participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization (except hospitalization for elective procedures for a pre-existing condition that has not worsened, admission to a nursing home or rehabilitation facility, or social admissions)
- Results in persistent or significant disability or incapacity (substantial disruption of the ability to conduct normal life functions; it does not include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma [eg, sprained ankle] that may interfere or prevent everyday life functions but do not constitute a substantial disruption)
- Is a congenital anomaly or birth defect (ie, an adverse outcome in a child or fetus of a female participant exposed to the study product)
- Important medical events that do not meet any of the above criteria may be considered serious when, based upon appropriate medical judgment, they jeopardize the participant and may require intervention to prevent one of the other outcomes listed in this definition

7.2 Method of Detecting AEs and SAEs

Recording, evaluating, and assessing causality of AE and SAE methods and the procedures for completing and transmitting SAE reports are provided in Section 11.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

7.3 Performing Adverse Events Assessments

The Investigator is responsible for monitoring of participants for AEs/SAEs over the assessment period as specified in the Schedule of Events (Table 4). Each AE/SAE will be evaluated as described in Section 7.4.1 and Section 7.4.3. Treatment of any AE/SAE will be managed by the participant's healthcare provider. If any aspect of evaluation or treatment of an AE/SAE is performed by a physician who is not part of the study team, the Investigator will ensure that the appropriate party is contacted as promptly as possible to collect the relevant information and documentation to complete the protocol-required assessment of each AE/SAE.

7.4 Timing

The Sponsor's medical monitor will have read-only access to the AEs recorded within the eCRF. All SAEs must be reported within 24 hours of disclosure/discovery. All SAEs will be recorded and reported to the Sponsor or designee immediately via automatic notification procedures from the eCRF and under no circumstance should this not exceed 24 hours. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Adverse events and SAEs will be collected from the start of study treatment until the Follow-Up assessment as specified in the Schedule of Events (Table 4). Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of eCRF, not on the AE section.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

7.4.1 Reportability

The Investigator, CRO medical monitor and Sponsor's medical monitor are responsible for determining whether an event should be classified as serious. If in the opinion of the Investigator, the AE meets the criteria of an SAE, the SAE is to be reported to the Sponsor or Sponsor's medical monitor designee. All SAEs, regardless of causality, must be reported. SAEs must be reported from the time the Informed Consent Form (ICF) is signed until 30 days after the last dose of study drug. SAEs considered related to study drug that occur after the specified Follow-up period should also be reported.

For expedited reporting purposes, SAEs assessed as Definitely Related, Probably Related or Possibly Related by the Investigator will be considered as "Related" events, while those assessed as Not Related will be considered as "Not related" events. These processes will be delineated in the CRO prepared, Safety/Medical Monitoring Plan, in consultation with sponsor's medical monitor prior to study.

7.4.2 Severity

The intensity (severity) of each AE will be assessed using the guidelines below:

- **Mild**: Does not interfere with usual activities, easily tolerated, requires no treatment.
- **Moderate**: Somewhat interferes with usual activities, may cause discomfort, may require treatment.
- **Severe**: Significantly interferes with usual daily activities, may be incapacitating, may require treatment.

Severity of an event is not the same as seriousness. Regardless of severity rating, an AE is considered serious if it meets the criteria for an SAE.

7.4.3 Relationship between Adverse Event and Study Drug

The relationship between the AE and study drugs (ie, separate assessments for relationship to study treatment) will be determined as one of the following:

- Definitely Related
- Probably Related
- Possibly Related
- Not Related

For analysis purposes, AEs assessed as Definitely Related, Probably Related or Possibly Related by the Investigator will be considered as "Related" events, while those Not Related will be considered as "Not related" events.

Causality will be determined based on clinical judgment and the following guidelines:

Definitely Related:

The event followed a reasonable temporal sequence from the time of study medication administration and/or followed a known response pattern to the study medication and could not have been produced by other factors such as the subject's clinical state, therapeutic intervention, or concomitant therapy and either occurred immediately following study medication administration or improved on stopping the medication.

Probably Related:

The event was reasonably temporally related to study medication administration, was consistent with known effects of the study medication, improved upon withdrawal of the study medication, and was unlikely explained by another etiology.

Possibly Related:

The event was reasonably temporally related to study medication administration which follows a known or expected pattern of the suspected medication but could be explained by another etiology.

Not Related:

The event was clearly due to extraneous causes (e.g., diseases, environment), which was to be specified if known; or the event was most likely produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant therapy and did not follow a known response pattern to the study medication.

The CRO Medical Monitor will review the Investigator's assessment of causality and may agree or disagree. Both the Investigator's and CRO Medical Monitor's assessments will be recorded. The CRO Medical Monitor will have the final say in determining the causality.

7.4.4 Adverse Drug Events

Adverse drug events (ADE) may be expected to be generally associated with irritation of the nasal mucous membranes in both NONS and placebo treated participants. These events may include headache, pharyngitis, epistaxis, nasal burning/nasal irritation, nausea/vomiting, asthma symptoms, and cough.

7.4.5 Expectedness

An unexpected AE is one of a type not identified in nature, severity or frequency in the current Investigator's brochure. Expected events may include upper respiratory irritation-like symptoms due to use of the nasal spray.

7.5 Adverse Event and Concomitant Mediations Coding

Adverse events will be coded using the current MedDRA dictionary and Concomitant Medications using the current WHODrug dictionary.

7.6 Reporting Serious Adverse Events and COVID-19 Reporting

7.6.1 Serious Adverse Event and Suspected Unexpected Serious Adverse Reaction Reporting

All SAEs that occur from the time of participant enrollment until the end of the study (whether drug related or not) will be documented on an initial SAE form. All SAEs will be reviewed by the Investigator and reported to within 24 hours or specified by regulatory and IRB for SAE reporting.

If only a partial SAE report is available, preliminary information will be documented on an SAE form, reviewed by the Investigator, and reported within 24 hours or specified by regulatory and IRB for SAE reporting. When additional relevant information is available, this information will be submitted on a new SAE form within 24 hours.

Refer to the Safety Management Plan for SAE reporting methods.

Reporting of SAEs to the IRB/IEC will be performed by the Investigator in accordance with the standard operating procedures and policies of the IRB/IEC.

The Sponsor will report SUSARs ([serious adverse drug reactions] that are not identified in nature, severity or frequency in the risk information set out in the Investigator's brochure or on the label of the drug) to the Regulatory Authorities (eg, HC/FDA, or other applicable relevant local/national regulatory authorities) in accordance with regulations (e.g., C.05.014(1)) and related guidance.

7.6.2 Study Sponsor Notification of Adverse Event Outcome by Investigator

Any SAEs, regardless of causality, must be reported to the Sponsor or Sponsor's designee within 24 hours of the Investigator or any staff's knowledge of the event.

Within 24 hours after receipt of additional relevant information, the Investigator must provide Follow-up information on the SAE along with any additional relevant documentation (eg, test results, physician narrative, discharge summary, autopsy findings) that will assist in the understanding of the event.

7.6.3 Institutional Review Board/ Independent Ethics Committee Notification

The Investigator must report all SAEs (including Follow-up information) to the IRB/independent ethics committee (IEC) in accordance with IRB/IEC requirements. Copies of each report and documentation of IRB/IEC notification and receipt will be kept in the local Regulatory files.

7.6.4 Regulatory Reporting Requirements for Serious Adverse Events

- Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/ IEC, and Investigators.
- For all studies, except those utilizing medical devices Investigator safety reports, must be prepared for SUSAR according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an Investigator safety report, describing a SAE or other specific safety information (e.g., summary or listing of SAEs), from the Sponsor, will review and then file it along with the Investigator's Brochure. He or she will then notify the IRB/IEC, if appropriate according to local requirements.

7.6.5 COVID-19 Health Authority Notification

Health Canada/FDA, and other relevant local/national regulatory authorities must be notified of any participant who contracts COVID-19 during the study (if applicable).

7.7 Treatment-Emergent Adverse Events

Information regarding the occurrence of treatment-emergent AEs (TEAEs) will be captured throughout the study and verified at each telehealth assessment. A description of the AE, duration (start and stop dates and times), severity, outcome, treatment and relation to study medication will be collected.

7.8 Adverse Events of Special Interest

- COVID-19 related symptoms or diagnosis
- Non-compliance for any reason
- Complaints or unanticipated problems associated with the manual pump and spray bottle or use of these, especially malfunction of the manual pump spraying apparatus or a deterioration in its effectiveness, or inadequacy of the directions for use

7.9 Adverse Event, Serious Adverse Event, and Adverse Events of Special Interest Follow-Up

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent assessments/contacts. All SAEs, and AEs of special interest will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to Follow-Up (Section 7.12.3).

7.10 Pregnancy Reporting

Should a female participant or female partner of a male participant become pregnant at any time during the study, the Investigator must notify the Sponsor within 24 hours after learning about the pregnancy. The Sponsor will request Follow-up information regarding the course of the pregnancy and its outcome. Voluntary consent must be obtained from a female participant or female partner of a male participant (and/or legally authorized representative if the participant is mentally incompetent or physically incapacitated) before any pregnancy-related follow-up is obtained.

Pregnancy, in and of itself, is not considered an AE, unless there is suspicion that the study product may have interfered with the effectiveness of a contraceptive medication or method. However, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE and will be followed accordingly. A spontaneous abortion is considered to be an SAE.

7.11 Concomitant Medication Assessments

Concomitant medication usage for a participant will be recorded for the period starting on Day 1 and continuing throughout the study, including medications administered for management of any AEs as described in Table 4.

Concomitant medications to be recorded include prescription and non-prescription drugs, including vaccines (including all previous SARS-CoV-2 vaccine doses), vitamins, herbal supplements, and recreational drugs.

Non-pharmacologic therapies should be recorded if relevant to a reported AE.

7.12 Safety Follow-Up

Safety Follow Up will be conducted on-site or by the qualified medical provider by telephone, as described in the Schedule of Events (Table 4).

7.12.1 Removal of Participants from the Trial or Study Treatment

The Investigator may withdraw a participant from the study for any of the following reasons:

- A protocol violation occurs,
- A serious or intolerable adverse event occurs,
- A clinically significant change in a laboratory parameter occurs,
- The Sponsor or Investigator terminates the study, or
- The participant requests to be discontinued from the study.

A participant may choose to discontinue study treatment and/or withdraw from the study at any time. Participants who become pregnant during the study are to be withdrawn from study treatment. In the absence of a medical contraindication, every effort should be made by the Investigator to encourage participants who prematurely discontinue treatment to remain in the study as appropriate and, at a minimum, follow the participant for safety data collection.

7.12.1.1 Discontinuation from Study Drug Treatment

Participants may discontinue for the following reasons: any study drug related AEs that in the opinion of the Investigator, makes it unsafe for the participant to continue; any study drug related AE that repeats upon re-challenge.

A participant may be discontinued prematurely from study drug treatment for the following reasons:

- AE (including hospitalization for COVID-19/sequalae)
- Withdrawal by participant (specify reason in the eCRF)
- Lost to Follow-up
- Pregnancy

- Physician decision (ie, Investigator decision – specify reason in the eCRF)
- Sponsor decision (specify reason in the eCRF)
- Noncompliance with study protocol
- Other (specify reason in the eCRF)

7.12.1.2 Discontinuation from Study

A participant may be discontinued prematurely from the study (in its entirety) for the following reasons:

- AE (including hospitalization for COVID-19/sequalae)
- Withdrawal by participant (specify reason in the eCRF)
- Lost to Follow-up
- Death
- Physician decision (ie, Investigator decision – specify reason in the eCRF)
- Sponsor decision (specify reason in the eCRF)
- Noncompliance with study protocol
- Other (specify reason in the eCRF)

If a participant is withdrawn because of an adverse experience, the participant will be followed and treated by their health care provider (with documented follow-up) until the abnormal parameter or symptom has resolved or stabilized.

If, for any reason, a participant does not complete the study, the reason will be entered on the eCRF. All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. The Investigator must record the reason for the early termination and document it in the eCRF.

7.12.1.3 Data Collection and Follow Up for Withdrawn Participants

If a participant is discontinued prematurely from treatment or from the study, the reason for discontinuation will be collected in the eCRF.

For participants who discontinue from the study for reasons other than withdrawal of consent, lost to Follow up, or death, a reasonable effort should be made to complete certain study procedures at or near the time of discontinuation.

Any AEs that are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements.

If a participant is lost to Follow-up, every reasonable effort must be made by the study site personnel to contact the participant and determine the reason for discontinuation/withdrawal.

7.12.2 Participant Replacement

Participants who withdraw from the study will not be replaced.

Participants who are withdrawn due to a positive COVID-19 infection prior to completing <2 days (48hrs) on study may be replaced at the discretion of the investigator.

7.12.3 Lost to Follow-Up

A participant will be considered lost to Follow-Up if they repeatedly fail to attend scheduled visits/contacts/telehealth assessments and are unable to be contacted by the study site.

The following actions will be taken if a participant fails to attend a scheduled visit/contact/telehealth assessment:

- The investigator/site/regional center must attempt to contact the participant and reschedule the missed assessment(s) as soon as possible. They must counsel the participant on the importance of maintaining the assigned assessment schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to Follow-Up, the Investigator or designee (i.e. regional call center) must make every effort to regain contact with the participant/emergency contact (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study.

7.13 Study Stopping Rules

A formal plan for interim data monitoring for futility will be established. Review of serious, unexpected, and related AEs by the CRO Medical Monitor, Data Safety Monitoring Board (DSMB), IRB, or HC/ FDA or applicable relevant local/national regulatory authorities may also result in suspension of further study agent administration. Health Canada/FDA/IRB/or local/national regulatory authority and study Sponsor(s) retain the authority to suspend additional enrollment and study agent for the entire study, as applicable.

7.14 Appropriateness of Measurements

All assessments used in the study are considered standard (ie, widely used and generally recognized as reliable, accurate, and relevant).

8 CLINICAL TRIAL MATERIALS

8.1 Study Treatment Supplies

8.1.1 Nitric Oxide Nasal Spray and Placebo

Supplies of NONS and placebo are described in Table 1.

Table 1 Identity of Investigational Product

Study Drug	Formulation
Nitric oxide spray (supplied by the Sponsor)	12.5 mL formula A in saline in one chamber, and 12.5 mL formula B in saline in the other chamber of the dual chamber nasal spray device which delivers a metered dose
Placebo (supplied by the Sponsor)	Sterile 0.9% Sodium Chloride, USP

8.2 Packaging and Labeling

The drug will be prepared and packaged in an ISO 13485 and GMP registered facility. The NONS solution will be prepared in three 25 mL bottles of manual pump nasal spray bottles with the labeled statement according to local regulatory guidelines for labeling “for investigational use only” with minimally the lot/batch number, expiry date, and storage conditions.

8.3 Distribution and Supply Management

Regional distribution and supply management is a critical piece to the overall success of the study. Distribution and supply management following participants’ randomization will be completed in accordance with the Supply Management Plan. The Sponsor in conjunction with support vendors, as applicable, will detail how study supplies are received, shipped and returned to sites for both remote and on-site enrolled participants. The Sponsor or designee (ie, CRO or supply management and support vendor(s)) will be responsible for maintaining this document throughout the course of the study.

For more details, refer to the Supply Management Plan.

8.4 Storage and Accountability

8.4.1 Study Treatment Storage

Study treatment provided to participants will be stored, dispensed, and recorded in accordance with this protocol, Pharmacy Manual, Supply Management Plan and other multicenter operational procedures document for this study including participant instructions. Only participants enrolled in the study may receive study treatment.

All study treatment should be stored at temperatures ranging from 2°C to 40°C (35°F to 104°F) and protected from flames and combustible materials. Study medication should be kept out of the reach of children.

8.4.2 Study Treatment Accountability

The Investigator or his/her designee will maintain accurate records of the receipt of all study treatment. In addition, accurate records will be kept regarding when and how much of each study treatment is dispensed, used and unused by each study participant and documented in the eCRF.

8.5 Investigational Product Retention at Sponsor

At the conclusion of the study, any unused or opened study treatment bottles will be returned to the Sponsor or designee for accountability tracking (refer to the Supply Management Plan). The Sponsor should not dispose of any used or unused study drug until drug accountability is performed by the Sponsor or its designee.

8.6 Preparation of Study Treatment

There is no preparation of the study treatment required.

8.7 Use of the Spray Bottle

In the event of a malfunction of the spray bottle, or the participants misplace the spray bottle, participants should contact the regional study center for further instructions.

At the end of the study, or at the time of participant discontinuation or early termination, nasal spray bottles will be returned to the Sponsor as described in the MOP/IOP.

Participants will complete the spray bottle PRO on Day 1 and Day 5, documenting their perception of the ease of use of the spray bottle (Appendix 3).

9 PLANNED STATISTICAL METHODS

9.1 General Considerations

A detailed statistical analysis plan will be developed separately and finalized prior to randomization of the first participant. Deviations from the protocol-planned analyses will be noted.

The statistical analysis of the data obtained from this study will be performed using SAS® Version 9.4 or higher. All statistical tests will be performed at the 0.05 (two-sided) significance level, unless otherwise noted.

The data collected in this study will be documented using summary tables and participant data listings. Continuous variables will be summarized using descriptive statistics, specifically the number of observations, mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized by frequencies and percentages. The analysis of both efficacy and safety parameters will be conducted for each treatment group separately. Active treatment will be compared against placebo.

Baseline characteristics will be summarized using descriptive statistics or frequencies and percentages, as appropriate. No statistical hypothesis tests will be performed on these characteristics.

9.2 Determination of Sample Size

For an annualized infection rate of 3% in the placebo arm (daily incidence = 0.000075), and assuming a true hazard ratio of 0.15 implying protective effect of NONS equivalent to a vaccine efficacy of 85%, a sample size of 11,480 participants randomized 1:1 (NONS:Placebo) and available in the modified intent-to-treat (mITT) population will provide 90% power to demonstrate superiority of NONS to placebo in preventing COVID-19 infections reported through 28 days, based on Cox regression model and a 2-sided 0.05-level test (Schoenfeld 1983). The expected number of events in the two arms is 2 and 10 for the NONS and placebo groups respectively. This further assumes 25% censoring during the 28-day period (1.05% loss per day).

Sample size calculations conducted under version 1.0 of the protocol (dated April 27, 2021) used estimates of annualized case rates as listed in Table 2. At that time, the recent estimated annualized infection rate based on confirmed cases ranged from 2.2 through 3.9% in select Canadian provinces, though there was considerable variability, and these rates underestimate the true infection rates considering those untested. The US and other nations may have substantially higher infection rates and require fewer subjects to be randomized.

In particular, for the Czech Republic, Slovakia, and Sri Lanka, the reported annualized rates are between 0.14% and 8.23%, 0.07% and 7.27%, and 1.48% and 6.18% respectively over the period of May 1, 2021, to July 31, 2021. We are therefore likely to have sufficient power to conduct this study in those countries.

The maximum number of participants who will be randomized is 13,000, in order to achieve a maximum of 11,480 participants initiating their randomized treatment assignment and entering the mITT population. If the infection rate is higher than assumed here, enrollment and randomization will be closed if the total number of COVID-19 events in the mITT population exceeds 18 cases, 50% more than the number expected for 90% power. This inflation accounts for the increased infection rate and the consequent increased risk of infection between the time of randomization and time of initiation of study treatment due shipping times.

Table 2 Daily COVID-19 Counts

Population	**Estimated annualized rate in February 2021 (through 25FEB2021**	**Equivalent daily counts based on study assumption in control arm**		**Days Daily Confirmed case counts exceeded 3% / 4% annualized rate 1FEB-25FEB2021**
		3% annualized rate	**4% annualized rate**	
Manitoba	2.2%	104	138	4 / 0 Days
Alberta	2.8%	328	437	12 / 1 Days
British Columbia	3.2%	380	507	21 / 3 Days
Quebec	3.9%	637	849	25 / 15 day
Ontario	2.9%	1101	1468	11 / 4 days
Canada (all provinces)	3.0%	2819	3759	20 / 2 days
United States	> 4%	24,615	32,820	25 days

Source: https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection.html

9.3 Analysis Populations

Analysis populations are Randomized participant, mITT, Per-Protocol (PP), and the Safety population (all participants receiving any dose of treatment), the Per Protocol population (received drug and had no major documented protocol violations), defined as in

Table 3.

Table 3 Populations for Analysis

Population	Description
Randomized	All participants randomized regardless of adherence to treatment or study status, analyzed as randomized.
mITT	All participants in the Randomized population who received at least one dose of the test article (NONS or placebo), analyzed as randomized.
Daily Use	All participants in the mITT set who documented at least one self-administered NONS dose per day on at least 25 of 28 expected dosing days or 90% of days while on study treatment. Analyzed as treated
Per Protocol	All participants in the mITT set who documented self-administered NONS on at least 25 of 28 expected dosing days or 90% of days and 80% of expected doses while on study treatment. Analyzed as treated.
Safety	All randomized participants who receive at least 1 dose of the study intervention.

Abbreviations: mITT=modified intent-to-treat; NONS=nitric oxide nasal spray.

9.4 Demographics and Baseline Characteristics

Demographic data will be provided in listings and summary tables with descriptive statistics. No statistical hypothesis tests will be performed on these characteristics.

9.5 Analysis of Efficacy

9.5.1 Primary Efficacy Analysis

The primary efficacy objective is to demonstrate that NONS product reduces the risk of COVID-19 infection. The associated null hypothesis is that the hazard ratio (HR) of NONS relative to placebo is greater than 1, or H_0: HR $\geq$ 1, or equivalently in terms of a vaccine efficacy, H_0: VE $\geq$ 0 where vaccine efficacy is calculated as VE = 100% x (1-HR). The HR will be estimated using a Cox proportional hazard regression model with a fixed effect only for treatment arm. Due to the small, expected event rate (see the Section 9.2), incorporation of additional fixed effects may not be possible. However, as a sensitivity analysis, a stratified Cox model using randomization strata ($\geq$ 65, <65 at increased risk, < not at increased risk) will also be fit. Similarly, age-group ($\geq$/<65) and region-specific models will be fit, aggregating adjacent provinces, states, or larger geographies to enable model convergence and evaluate, to the extent possible, whether differences exist.

The primary analysis will be executed in the mITT data set and count all cases occurring starting from the day after participants begin administering study treatment.

For all models, a 2-sided score-based 95% confidence interval (CI) and 2-sided p-value for the effect of NONS will be calculated.

The estimand of the primary analysis is a treatment policy strategy and will consider intercurrent events as censored. Specifically, participants who stop administering NONS for any reason, stop submitting data to the electronic patient-reported outcomes (ePRO) system, do not submit paper diaries, or are unreachable by telephone contact, will be censored on the day of their last ePRO clinical status submission or last available documented day on paper diary (ie, the last day confirming no positive COVID-19 test). Participants who submit COVID-19 test results following Day 28, will be censored at Day 28. Participants submitting a COVID-19 test result on or prior to Day 28 will be considered to have reached the primary endpoint on the day that the specimen (ie, nasal swab) is collected, not the date of the test. Positive test results obtained within 48 hours of randomization will be considered infected at randomization and not counted as events.

The Cox model will be fit to the following sets (if different) as sensitivity analyses:

- mITT set counting all cases after randomization,
- PP set counting cases after first administration,
- PP set counting all cases after randomization, and
- Randomized set counting all cases after randomization

The primary analysis will be repeated in the following subgroups:

- Subjects unvaccinated against SARS-CoV-2 prior to randomization
- Subjects partially vaccinated against SARS-CoV-2 prior to randomization
- Subjects fully vaccinated against SARS-CoV-2 prior to randomization
- Subjects who are fully vaccinated and have received a booster shot against SARS-CoV-2 prior to randomization
- Subjects who become partially vaccinated against SARS-CoV-2 during the course of the study
- Subjects who become fully vaccinated against SARS-CoV-2 during the course of the study
- Subjects who receive a booster vaccine against SARS-CoV-2 during the course of the study

Specific procedures to account for missing data, unused or spurious data and criteria for termination will be delineated in the SAP. Procedures for reporting any deviation(s) from the original statistical plan will be described in the SAP and justified (if needed) in the final clinical study report.

9.5.2 Secondary Efficacy Analysis

For the secondary objective of prevention of medical intervention for COVID-19, the secondary endpoint will be executed similarly to the primary efficacy analysis above.

Participants who are hospitalized for COVID-19-related symptoms, but who test negative for COVID-19 will not be considered to have met the endpoint. The time of the endpoint will be considered the study day at which the participant was hospitalized or the day at which the ER/ED visit occurred even if a COVID-19 positive result was obtained before or after the day of the event.

9.5.3 Exploratory Analysis

Device use questions to support the human factors study of the delivery device will be performed as an exploratory analysis as described in the SAP. Any AEs, protocol deviations, or compliance issues identified in this study will be included in the primary analysis of this study and will be detailed in the main SAP.

9.5.4 Analysis of Safety

All safety analyses will be performed in the Safety Population.

The primary analysis of safety will include counts and proportions of participants with Aes and SAEs summarized by MedDRA System Organ Class (SOC) and Preferred Term (PT) and cross-tabulated by severity, relationship to study treatment, treatment received, and overall. Exact Clopper-Pearson 95% confidence intervals for proportions may be constructed.

All AEs and SAEs will be reported in a listing and events of interest will be itemized and listed separately.

9.6 Interim Analysis

Given the small number of expected events, there will be no interim analysis for efficacy. As described in Section 9.2, the enrollment may be closed when 18 cases are accumulated, but this will be considered the final analysis, not an interim analysis.

After every approximately 2,000 participants from the mITT cohort complete the study, the aggregated COVID case-rate will be used to calculate the projected number of cases for the full sample size of 11,480. The study may be considered futile if fewer than 10 cases are projected to be observed for the full sample size.

10 QUALITY CONTROL AND ASSURANCE

10.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018)
- International Council for Harmonisation (ICH) E6 (R2) Guideline for Good Clinical Practice
- Food and Drug Regulations Part C, Division 5 Drugs for Clinical Trials Involving Human Participants
- Health Canada ‘s Management of clinical trials during the COVID-19 pandemic: Notice to clinical trial Sponsors
- Regulations of all other jurisdiction where the trial is undertaken, eg, with respect to the EU, all applicable laws and regulations of a member state where the trials is undertaken, especially those transposing and implementing Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Or the Regulation EU No 536/2014 of the European Parliament and of the Council on clinical trials on medicinal products for human use should it become effective in term of the present Agreement. Plus the Regulation (EU) 2016/679 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation), and if in the United States, regulations 21 Code of Federal Regulations (CFR) 50, 56 and 312.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures

Providing oversight of the conduct of the study at the site and adherence to requirements of Food and Drug Regulations Part C, Division 5 Drugs for Clinical Trials Involving Human Participants, in particular the undertakings and practices described in: C.05.012(3)(f) (Qualified Investigator Undertaking) and C.05.010 concerning Good Clinical Practices.

Therapeutic Product's Directorate Guideline for Good Clinical Studies, Guidance Document: Part C, Division 5 of the Food and Drug Regulations "Drugs for Clinical Trials Involving Human Participants" (GUI-0100) – Summary or all other applicable local regulations e.g. US regulations 21 CFR 312. 60-68.

10.1.1 Data Protection

- Participants will be assigned a unique study number. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- Only participant data will be analyzed during the study.
- The following emergency contact data will be collected: informed consent, name, and telephone number.
- The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the ICF.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.2 Dissemination of Clinical Study Data

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. The Investigator is obligated to keep data pertaining to the study confidential. Archiving per country specific requirements (eg, 25 years in Canada).

10.1.3 Auditing and Inspecting

The study may be evaluated by representatives of the Health Canada, and where applicable, other government agencies, national health agencies, and national health authorities, who also will be allowed access to study documents. The Investigator and regional centers should promptly notify the Sponsor when any inspections are scheduled, as well as providing the results of any unscheduled investigations performed by any regulatory authorities.

The Investigator and regional centers will permit study related monitoring, audits, and inspections by the IRB/EC, the Sponsor, government regulatory bodies, and compliance and quality assurance groups of all study related documents (eg, source documents, regulatory

documents, data collection instruments, study data, etc.). The Investigator will ensure the capability for inspections of applicable study-related facilities. All authorized personnel, including health authority inspector(s), Sponsor and designees, Study/CRO Medical Monitor(s), Data Monitoring Committee (DMC) members, and auditor(s) will be given direct access to source data and documentation (eg, medical records, laboratory results, etc.) for source data verification, provided that participant confidentiality is maintained in accordance with local requirements.

Participation as an Investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance and quality assurance offices.

11 ADMINISTRATIVE CONSIDERATIONS

11.1 Institutional Review Board (IRB) or Independent Ethics Committee (IEC) Approval

The Investigator, sub-Investigator, and regional centers will provide the ethics review board (eg, IRB or IEC) with the study protocol, the ICF, all written materials for the participant, and all advertisements used for participant recruitment. No participants are to be enrolled in the study until the IRB/IEC provides written approval of the protocol and the ICF, and not until approval documents have been sent to the Investigator and copies received by the Sponsor. All reviews will be in accordance with GCP. The IRB will be notified of any SAEs reported on the study. Appropriate reports on the progress of the study will be made by the Investigator to the IRB/IEC and to the Sponsor in accordance with the applicable government regulations, and in agreement with the policy established by the Sponsor.

11.2 Ethical Conduct of the Study

The study will be conducted in accordance with GCP requirements as described in the current revision of the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines and all applicable regulations, including the current Canadian Food and Drug Regulations Part C, Division 5 in particular C.05.010, US Code of Federal Regulations (CFR), Title 21, Parts 50, 54, 56, and 312, and Title 45, Part 164. Compliance with these regulations and guidelines will also constitute compliance with the ethical principles that have their basis in the Declaration of Helsinki and as outlined in the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans – TCPS 2 (2018). This study will also be carried out in accordance with local legal requirements in each participating country.

11.3 Participant Information and Consent

Each participant will be provided a written ICF (electronic or hard copy), describing this study and providing sufficient information to make an informed decision about participation in this study. The participant will be given opportunities to discuss and review the study requirements with a regional representative for their location. Copies of completed consents, will be provided back to the participant (digitally or hard copy) based on the email address provided by the participant or in-person during the on-site visit.

A copy of the proposed ICF must be submitted to the Sponsor and/or designee for review prior to submission to the reviewing IRB/EC. The ICF must be approved by the IRB/EC and must contain all elements required by federal, provincial, state, local, and institutional regulations or requirements. Canadian institutions must comply with all applicable federal and provincial privacy legislation (see Section 11.5).

Institutions in the US must be in compliance with Health Insurance Portability and Accountability Act of 1996 with regard to obtaining authorizations for use and disclosure of Protected Health Information. Local privacy regulations must be followed, including the

General Protection Data Regulation (GDPR) applicable in the EU. For Canada, see Section 11.4 regarding legal obligations for privacy and confidentiality of personal information.

The study, including its goals, methods, expected benefits, and potential hazards will be completely explained to each prospective study participant. It will also be explained to participants that they are free to refuse entry or to withdraw from the study at any time without prejudice to future treatment. Voluntary informed consent must be obtained from each eligible participant (and/or legally authorized representative if the participant is mentally incompetent or physically incapacitated) before any protocol-defined procedures are performed.

The participant's willingness to participate in this study will be documented on the IRB/EC approved ICF which must be signed and dated by the participant or legally acceptable surrogate, and the physician Investigator (Principal or sub-Investigator). The Investigator will keep the original ICF, and a copy will be given to the participant. The process for obtaining informed consent should also be noted in the participant's source documentation.

11.4 Patient Confidentiality

The Investigator, sub-Investigator, and regional centers must assure that the privacy of the participants, including their identity and all personal medical information, will be maintained at all times. In the eCRFs and other documents (eg, laboratory reports, etc.) submitted to the Sponsor, participants will not be identified by name, but by an identification code (eg, participant identification numbers). Remote monitoring may involve either a redacted source document or remote review of unredacted source document, per CRO and/or institutional polices. All Non-Canadian institutions must comply with all applicable national and regional privacy legislation. Canadian institutions must comply with all applicable federal (Personal Information Protection and Electronic Documents Act) and provincial privacy legislation eg,:

- BC Personal Information Protection Act, Freedom of Information and Protection of Privacy Regulations, BC eHealth Act,
- Alberta Personal Information Protection Act
- Ontario Personal Health Information Protection Act
- Quebec Loi sur la protection des renseignements personnels dans le secteur privé

Personal medical information may be reviewed for the purpose of verifying data recorded in the CRF by the Study/CRO Medical Monitor, Sponsor or designee, or regulatory authorities. Personal medical information will always be treated as confidential. Personal data is also protected in accordance with the personal data privacy legislation in each participating country, per the General Protection Data Regulation (GDPR) applicable in the EU.

11.5 Study Monitoring

The Investigator/regional Principal Investigator will allocate adequate time for remote study monitoring activities. The regional Investigator will also ensure that the Study Monitor, or

other compliance or quality assurance reviewer, is given access to all study-related documents and study related facilities.

The Study Monitor will review ICFs and eCRFs and compare them with source documents to verify adherence to the protocol, and to ensure complete, accurate, consistent, and timely collection of data. The Investigator will be asked to provide any missing information or to clarify any discrepancies found by the Study Monitor.

In addition to source document verification of CRFs and ICFs, the Study Monitor will review the Clinical Site Regulatory Binder that is to be maintained by the Principal Investigator.

Refer to the Study Monitoring Plan for source data verification (SDV) and remote monitoring requirements and frequency.

11.6 Case Report Forms and Study Records

All eCRFs will be completed according to eCRF completion guidelines. The Principal Investigator must electronically sign and date a declaration on the eCRF attesting to his/her responsibility for the quality of all data recorded and that the data represent a complete and accurate record of each participant's participation in the study. An electronic copy of the completed eCRF will then be sent to the Investigator to be retained for their records.

11.7 Data Monitoring Committee

The DMC will review participant safety data regularly (meeting at least biannually), and after 1,000 participants have completed 5 weeks of Follow-Up. The composition and policies of the DMC are described in the DMC Charter. If any safety concerns arise, these data will be conveyed to the DMC expeditiously. After review of the data, the DMC will make any recommendations regarding study conduct.

11.8 Protocol Violations/Deviations

Except for a change intended to eliminate an immediate hazard to a study participant, the protocol shall be conducted as described without any changes or deviations. Any change must be reported immediately to the Sponsor and to the IRB/EC and/or regulatory authority as required by guidelines or regulation.

11.9 Access to Source Documentation

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents including web-based documentation are to be filed at the regional centers.

Data reported entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available for all data collected in the eCRF.

Refer to the Monitoring Plan for SDV review and requirements.

11.10 Principal Investigator Sign-Off on Electronic Case Report Forms

Whomever completes the medical assessment and initial consent for the participant is responsible for reviewing and signing off on the eCRF at the end of the study.

11.11 Data Generation and Analysis

All participant data relating to the study will be recorded directly into the eCRFs.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRFs.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and remote monitoring techniques are provided in the Monitoring Plan.

11.12 Retention of Data

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained subject to applicable local regulations after study completion, eg, in Canada 25 years. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

11.13 Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities, e.g. US regulation 21 CFR Part 54, which also covers the spouse and each dependent child of the Investigator. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

11.14 Publication and Disclosure Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support

publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors (ICMJE) authorship requirements.

The Sponsor will adhere to the trials registration policy adopted by the ICMJE member journal. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is Sponsored by the National Library of Medicine.

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13 APPENDICES

Appendix 1 Schedule of Events

Table 4 Schedule of Events

Assessments	Screening [a] Baseline	Daily on TX	Early TX Day	Last Tx Day	Post-Tx Follow-up	
			Phone Assessment 1	Phone Assessment 2	Phone Assessment 3	ET
Visit Type (per specific country regulatory legislation)	**Remotely or On-Site**	**Remotely**	**Remotely**	**Remotely**	**Remotely or On-Site**	**Remotely or On-Site**
Study Day	**-7 to 1**	**1-35[d]**	**7+3**	**28 +2**	**End of Study 35±3**	
Participant Informed Consent (Virtual, if applicable/respective country) [b]	X					
Demographics (Virtual, if applicable/respective country)	X					
Medical History (Virtual, if applicable/respective country)	X					
Clinical Exam including Vitals (Virtual, if applicable/respective country)	X				X	X
Pregnancy Test (Urine) [c]	X				X	X
Concomitant Medications [d]	X	X	X	X	X	X
Randomization	X					
Study treatment instructions [e]	X		X			
COVID related symptom assessment [f]			X	X	X	X
NONS Self Administration [g]		X	X	X		
Questionnaire (online/paper) [h]		X[i]	X	X	X	X
Adverse Events		X	X	X	X	X
Study Drug Accountability/Compliance		X	X	X		X
Report if tested positive for COVID-19 infection		X	X	X	X	X
Report need for Hospitalization/ER Visit for COVID-19 related sequelae		X	X	X	X	X

Abbreviations: ER=emergency room; ET= early termination; NONS= nitric oxide nasal spray; Tx=treatment.

[a] Screening and baseline are to be taken within 7 days but may be taken at the same assessment. Post-Treatment Follow-up visits (end of study and ET) may be performed remotely. For countries requiring an in-person visit, these will be performed on-site, unless the participant is hospitalized, in quarantine or unable to visit the site for any reasonable cause as per the investigators' judgement; these visits may then be performed remotely and will not be considered a protocol deviation. Follow-up of all adverse events must be completed as described in the protocol Section 7.

[b] Participants will be asked to designate an emergency contact who will also sign informed consent. In the event the participant is hospitalized and cannot complete their treatment diary or questionnaire emergency contacts will be encouraged to provide symptoms on behalf of a participant in a COVID-19 symptoms list Diary (Appendix 2).

[c] Pregnancy test to be self-reported if remote visit is allowed per respective country regulatory legislation; however, results must be confirmed at Screening prior to enrolling the participant on the study.

[d] Excludes naturopathic products.

[e] For remote visits per respective country regulatory legislation, instructions on administering the study treatment will be provided via videocall on Day 1 and a check in call to confirm comfort with administration of the study treatment. For in-person visits, instructions will be given by the sites' staff with a confirmation of the participants' comfort with administration of the study treatment.

[f] Assessment for COVID-19 symptoms will be performed using the COVID-19 symptoms list (Appendix 2; Appendix 3).

[g] Dosing should start within 2 days of the baseline assessment.

[h] For the Spray Bottle Use Perception Questionnaire PRO, participants will only provide their feedback on the usage of the spray bottle on Day 1 and Day 5 (Appendix 3).

Appendix 2 COVID-19 Symptoms List

Centers for Disease Control COVID-19 Symptoms List

People with COVID-19 have had a wide range of symptoms reported – ranging from mild symptoms to severe illness**. Symptoms may appear 2-14 days after exposure to the virus**. People with these symptoms may have COVID-19:

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose
- Nausea or vomiting
- Diarrhea

This list does not include all possible symptoms. Centers for Disease Control (CDC) will continue to update this list as we learn more about COVID-19 (Centers for Disease Control and Prevention 2021).

Health Canada COVID-19 Symptoms List

Symptoms of COVID-19 can vary from person to person. They may also vary in different age groups. The more commonly reported Health Canada COVID-19 symptoms are similar to those reported in the Centers for Disease Control COVID-19 symptoms list. Children tend to have abdominal symptoms and skin changes or rashes. Symptoms may take up to 14 days to appear after exposure to COVID-19 (Health Canada 2021).

Appendix 3 Electronic Patient-Reported Outcomes Survey

COVID-NONS – 04

The complete language and content of all treatment (diary) and COVID-19 symptoms associated PRO questions are described in the Manual of Procedures (eCRF Section).

The daily questions address the participants' study medication dosing compliance (doses administered, bottle number in use, spray bottle mishaps/malfunction, etc.), treatment emergent adverse events (TEAE), changes to concomitant medications, and other general health-related changes. The specific daily symptoms and spray bottle use perception (Day 1 & 5 only) questions, include:

I. COVID-19 Symptom PRO Assessments (reported each day).

Over the last 24 hours did you have any of the following symptoms; if so, what was the severity of the symptom at its worst (Check best box/button for the symptom)?

1. Stuffy or Runny Nose	☐	None	6. Muscle or body aches	☐	None
	☐	Mild		☐	Mild
	☐	Moderate		☐	Moderate
	☐	Severe		☐	Severe
2. Sore throat	☐	None	7. Headache	☐	None
	☐	Mild		☐	Mild
	☐	Moderate		☐	Moderate
	☐	Severe		☐	Severe
3. Shortness of breath or difficulty breathing	☐	None	8. Chills or shivering	☐	None
	☐	Mild		☐	Mild
	☐	Moderate		☐	Moderate
	☐	Severe		☐	Severe
4. Cough	☐	None	9. Feeling hot or feverish	☐	None
	☐	Mild		☐	Mild
	☐	Moderate		☐	Moderate

		Severe			Severe
5. Low energy or tiredness		None	10. Nausea, or feeling like you wanted to throw up		None
		Mild			Mild
		Moderate			Moderate
		Severe			Severe

How <u>often</u> did you have the following 2 symptoms in the <u>last 24 hours</u>
(Check best box/ button for the number of times having the symptom)?

11. How many times did you vomit (throw up)		None	12. How many times did you have diarrhea (loose or watery stools)		None
		1 to 2 times			1 to 2 times
		3 to 4 times			3 to 4 times
		5 or more			5 or more

Rate your sense of <u>smell and taste</u> in the <u>last 24 hours</u> (Check best box/ button for the symptom)?

13. Sense of smell		Same	14. Sense of taste		Same
		Less			Less
		Smell Lost			Taste Lost

II. Spray Bottle Use Perception Questionnaire (Day 1 and Day 5 only)

Spray bottle 'experience' questions will be included on Day 1 (i.e. after one or more doses) and Day 5. The four-point Likert rating response options should be coded as -3, -1, 1, and 3 (most negative to most positive).

Spray Bottle Use Perception Questionnaire

Please answer the following questions about your experience with using the spray bottle (strongly disagree to strongly agree);

1. **The spray bottle was easy to operate?** Strongly Disagree; Disagree; Agree; Strongly Agree
2. **The tip of the spray bottle in the nose was comfortable?** Strongly Disagree; Disagree; Agree; Strongly Agree
3. **The smell of the spray was mild to none?** Strongly Disagree; Disagree; Agree; Strongly Agree
4. **The aftertaste of the spray was mild to none?** Strongly Disagree; Disagree; Agree; Strongly Agree
5. **The spray mist was gentle?** Strongly Disagree; Disagree; Agree; Strongly Agree
6. **The nasal irritation was minimal to none?** Strongly Disagree; Disagree; Agree; Strongly Agree
7. **The amount of spray that leaked out of the nose was minimal to none?** Strongly Disagree; Disagree; Agree; Strongly Agree

Appendix 4 Sponsor Signatures

Study Title:	Multicenter, randomized, double-blinded, placebo-controlled, phase 3 clinical efficacy study evaluating nitric oxide nasal spray (NONS) as prevention for treatment of individuals at risk of exposure to COVID-19 infection
Study Number:	COVID-NONS-04
Final Date:	08 December 2021

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed:_____ Date:_____
Christopher Miller, PhD, BA, RT
Chief Science Officer
SaNOtize Research and Development Corporation

Signed:_____ Date:_____
Gilly Regev, PhD
Chief Executive Officer
SaNOtize Research and Development Corporation

Appendix 5 Site Physician Investigator's Signature

Study Title:	Multicenter, randomized, double-blinded, placebo-controlled, phase 3 clinical efficacy study evaluating nitric oxide nasal spray (NONS) as prevention for treatment of individuals at risk of exposure to COVID-19 infection
Study Number:	COVID-NONS-04
Final Date:	08 December 2021

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Name (Print): _____

Title: _____

Address: _____

_____

Telephone #: _____

Signed: _____ Date: _____