APPENDIX II INTERVENTION SPECIFIC APPENDICES

C. INVESTIGATIONAL PRODUCT: USUAL CARE + SALINE

1. INTRODUCTION, RATIONALE AND PROTOCOL STRUCTURE

Many acute respiratory viruses, such as SARS-CoV-2, are primarily spread from person-to-person through aerosol or droplets that enter the body through orifices, such as the nose [1]. The angiotensin converting enzyme 2 (ACE-2) receptor is highly expressed in the nasal endothelium, and is believed to be an important portal for entry for SARS-CoV-2 virus into the cells of the upper airways [2]. SARS-CoV-2 is likely to upregulate ACE-2 on cell surfaces, binding to it and using it to facilitate cell entry [3]. Given that nasal carriage of acute respiratory viruses is an important portal for host cell entry, interventions that can reduce nasal viral load, thereby reducing spread to the upper airways and beyond, have the potential to ameliorate, or indeed avert, serious or systemic infection. Saline's topical action could change the conditions in the upper airways to make them more hostile to SARS-CoV-2 and other respiratory viruses.

Few studies to date have assessed the use of saline as an early treatment for acute respiratory viruses (see 'status of development of the IMP' section below). Given the mixed evidence of possible benefit with nasal saline from few studies with small sample sizes and of variable quality, further definitive evaluation of the intervention through a rigorous clinical trial may have merit. Given potential antiviral and or out-washing activity, saline may not be suitable as a pure placebo agent against which to evaluate other topical intranasal interventions. Furthermore, if proven to be more effective than Usual Care without topical intranasal saline, then this evidence could support the use of this cheap, safe, and scalable treatment.

2. OBJECTIVES

2.1 Primary objectives

As per master protocol.

3. STUDY DESIGN

3.1 Study Design

This IMP will be tested in a Phase IIb study.

3.2 Study Description

IMP initiation to occur within **3 days** of symptom onset.

3.3 Schematic diagram of study design

As per master protocol.

3.4 Duration of the study per participant

As per master protocol. IMP self-administration for 7 days.

4. STUDY POPULATION

4.1 Population (base)

As per master protocol.

4.2 Inclusion criteria

As per master protocol. Additional inclusion criteria for saline arm:

- Onset of symptoms within 3 days.
- For women of child-bearing potential*: prepared to use a highly effective method of contraception* or abstinence* for 30 days before and after terminating study medication intake.

* See master protocol.

4.3 Exclusion criteria

As per master protocol. Additional exclusion criteria for saline arm:

- Known to be currently pregnant or breastfeeding. Pregnancy should be ruled out by a negative urine pregnancy test for all women of child-bearing potential* prior to randomisation.
- Current or history of moderate to severe epistaxis or hereditary hemorrhagic telangiectasia.
- History of cerebral spinal fluid leaks via the sinuses/nose.
- Recent nasal fracture, nasal tumors, nasal masses, meningoencephalocele, and/or nasal surgery within the previous 2 weeks.
- Using any of the contradicted agents within 7 days before screening:
    - NO donors/derivatives: isosorbide dinitrate, isosorbide mononitrate, nitroglycerine/glyceryl trinitrate, nitroprusside, nicorandil.
    - Phosphodiesterase inhibitors: avanafil, sildenafil, tadalafil, vardenafil.
    - Guanylate cyclase activators: riociguat, linaclotide.
- Known glucose-6-phosphate-dehydrogenase (G6PD) deficiency.

* See master protocol.

4.4 Sample size calculation

The maximum sample size of 333 per arm has a one-sided error rate less than 2.5% and power around 90% for median time to recovery ranging from 12 to 6 days in the control group and a hazard ratio of 1.33. This assumes analysis using a Bayesian piecewise exponential model with weakly informative priors and interim analyses with early stopping rules for futility and superiority when 150 and 225 patients have been recruited to the control group and have been followed for 28 days. Early stopping rules for both superiority and futility are based on thresholds of the posterior distribution that have been justified using simulation (see M-SAP and section 10 below). Success is declared at maximum sample size if the posterior probability of superiority is greater than a final superiority threshold, which again is specified in the M-SAP and below in section 10.

5. STUDY TREATMENTS

5.1 Investigational Products

5.1.1 *Name and description of the IMP*

Saline is a saltwater solution (0.9% sodium chloride).

The IMP will be manufactured and supplied by SaNOtize Research and Development Corp. 25th Floor, 700 West Georgia Street, Vancouver, BC, Canada (manufactured at Glenmark Pharmaceuticals Limited Plot No. B-25, MIDC, Shendra Aurangabad 431154 Maharashtra, India). Myonex GmbH [Salfuzer 13/14 Aufgang A, 1.OG, 10587 Berlin, Germany] will label and distribute the IMP.*Status of development of the IMP*

Activity against SARS-CoV-2

Few studies have evaluated the use of intranasal saline as a treatment for COVID-19. These studies typically have small sample sizes and have often used saline as a control, rather than an intervention. In a three-armed, randomized clinical trial, 72 SARS-CoV-2 positive patients were randomly assigned to: (1) twice daily nasal irrigation with normal saline and Johnson & Johnson's baby shampoo (J&J/HTS); (2) twice daily nasal irrigation with normal saline (HTS); or, (3) no nasal irrigation (control). No significant difference was demonstrated between the J&J/HTS group and the HTS group, nor the control group, at any point from day 1 to day 21 [6]. In a pilot, placebo-controlled randomized clinical trial of povidone iodine (PV-I) nasal sprays (0.5% and 2.0% strength) versus saline nasal spray (0.9%), viral load reduced in all groups over time, however, there was no significant between group difference in viral load at day 3, assessed using nasal PCRs [(saline reference) 0.5% PV-I mean difference: -0.349 cycles/hour, 95% CI -1.584 to 0.886; 2.0% PV-I mean difference: -1.059 cycles/hour, 95% CI -2.318 to 0.201; n=35] [7].

In a pilot case-control study (n=140), consecutive patients testing positive for SARS-CoV-2 virus between December 2020 and February 2021 performed nasal irrigation with saline for 12 days [8]. Their symptoms, elicited through questionnaires, were compared with historical controls of SARS-CoV-2 infected patients from February-March 2020 [8]. Saline irrigation was associated with a *within-group* significant reduction in blocked nose (p=<0.0001), runny nose (p=<0.0001) and sneezing (p=0.010), whilst the control group was associated with a within-group reduction in blocked nose only (p=<0.0001) from baseline to day 10 [8]. No between group differences were reported.

Activity against other viruses

A pilot, open-label randomized trial of hypertonic saline nasal irrigation and gargling versus standard of care in 66 adults within two days of onset of upper respiratory tract infection (URTI) symptoms found that the intervention resulted in a significant reduction in: illness duration (1.9 days shorter, p=0.01); use of over-the-counter medications (36% reduction, p=0.004); household contact transmission (35% reduction, p=0.006); and, viral shedding (≥ 0.5 $\log_{10}$/day reduction, p=0.04) [9]. Participants grew a variety of viruses from their nasal passages, including rhinovirus, coronavirus, enterovirus and influenza virus [9].

119 adults with symptoms of a cold or acute sinusitis were randomly assigned to use either a hypertonic nasal spray (HNS) or a normal saline nasal spray (NS) three times a day, or received no intervention (control) [10]. No significant between-group difference was found in the duration of illness (HNS versus control p=0.24; HNS versus NS p=0.93), nor in the mean nasal symptom scores [10]. Slapak *et al.* conducted a randomized, open-label controlled trial of a nasal wash using a seawater solution versus usual care in 401 children (aged 6-10 years) in the outpatient setting with uncomplicated colds or influenza [11]. By visit two (up to three weeks after enrollment), parents of children receiving the intervention reported significantly less nasal secretion, less nasal breathing and reduced use of over-the-counter nasal decongestants, compared with controls (p<0.05). By visit four (up to 12 weeks post enrolment), parents of children receiving the intervention reported a significant improvement in health status of their children, compared with controls (p<0.05) [11].

5.1.2 Description and justification of dosage and route of administration

Saline will be administered intranasally six times a day [2 sprays each nostril, equivalent to 0.45 mL volume total per dose (4 sprays)], for seven days, using a droplet (0.115-0.130 gram) nasal spray.

Study medication should be administered upon awakening (Dose 1), then additional doses (Dose 2 to 5) approximately every 2-3 hours while awake. Doses should be separated by at least 1.5 hours. Preferably, the last dose to be administered (Dose 6) just before going to bed.

Administration guidance: Gently insert the bottle tip just into one nostril. Press on the other side of your nose with one finger to close off the other nostril. Aim slightly away from the center of your nose, point at the direction of the ear, and pump one spray. Sniff gently through the nose while applying, do not inhale deeply. Exhale through your mouth. Ensure that the spray is held vertically while spraying.

Saline dosing will follow the IMP schedule of previous randomized controlled trials (RCTs) with saline and nitric oxide nasal spray (NONS), i.e. India COVID-19 Phase 3 Treatment RCT, Bahrain COVID-19 Phase 3 Treatment RCT, and the revised Global COVID-19 Phase 3 Prevention RCT.

5.2 Additional considerations for trials involving a medical device

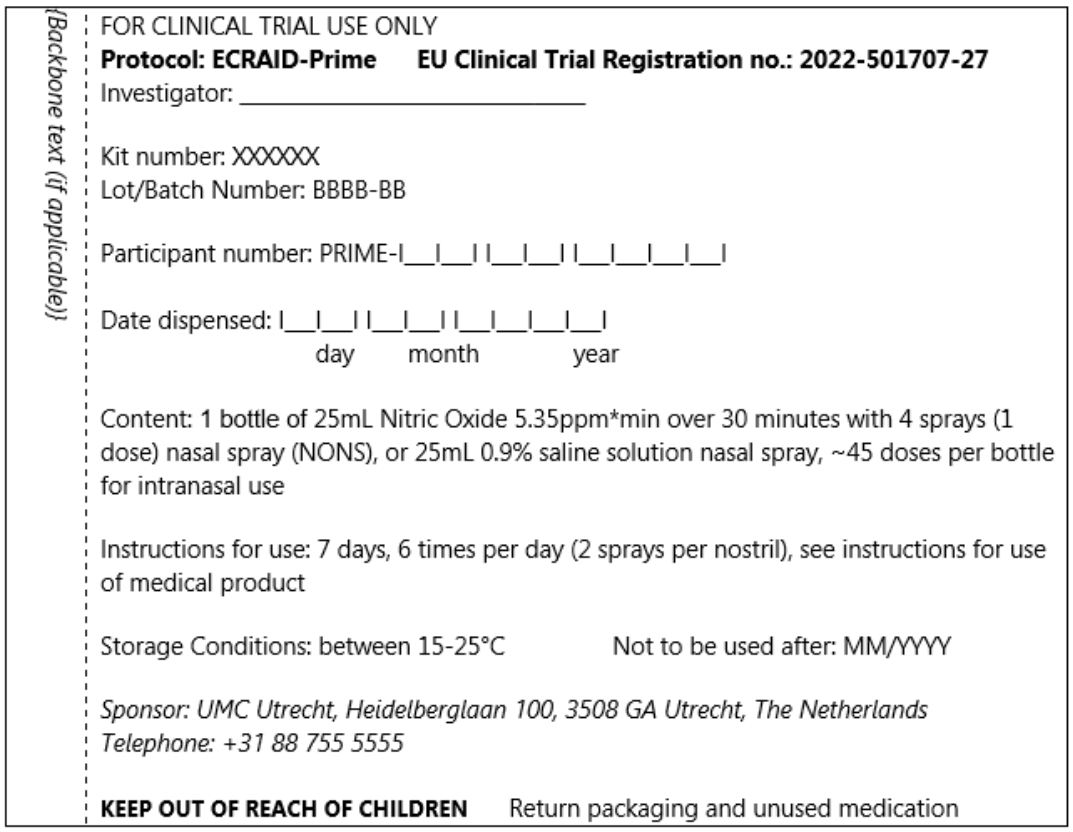
Not applicable.

5.3 Preparation and labelling of the Investigational Medicinal Products

Participants randomised to this treatment arm will be allocated an individual IMP pack according to the randomization number, including one 25 mL bottle, which is sufficient for seven days of administration.

Saline will be labelled in such a way that the double-blind design of the study is maintained. Labels on the IMP will contain all information required for regulatory (by country) and identification purposes.

Example of an English label:



6. OTHER TREATMENTS AND RESTRICTIONS

6.1 Concomitant therapy

6.1.1 Permitted medication

Participants should continue to take their usual prescribed medications throughout the study period, and can take symptomatic respiratory infection treatments e.g., antipyretics, simple analgesia and antitussives. These treatments will be permitted before and during the study. Usual care medications will be recorded for the duration of the study (e.g. antibiotic, antiviral, inhaled and pain/fever medication).

Participants requiring initiation of new medications or treatment for their respiratory infection during the seven days of IMP administration will be advised to contact the appropriate study staff personnel.

6.1.2 Prohibited medication

The following medications are prohibited for *7 days following* randomisation and for *within 7 days before* screening:

- NO donors/derivatives: isosorbide dinitrate, isosorbide mononitrate, nitroglycerine/glyceryl trinitrate, nitroprusside, nicorandil.
- Phosphodiesterase inhibitors: avanafil, sildenafil, tadalafil, vardenafil.
- Guanylate cyclase activators: riociguat, linaclotide.

Use of prohibited medications during the study will be documented as a protocol deviation. The decision about use of data from such participants is detailed in the statistical analysis plan.

6.1.3 Escape medication

As per master protocol.

6.2 Lifestyle restrictions

6.2.1 Contraception measures

Pregnant and breastfeeding women are excluded from this arm (because of the comparator IMP). See inclusion criteria and definitions in master protocol.

6.2.2 Other requirements

There are no other requirements.

7. TRACEABILITY, STORAGE, ACCOUNTABILITY AND COMPLIANCE

Saline should be stored between 15-25°C and should not freeze. Saline should be discarded on the expiration date printed on the bottle, or 60 days after first opening, whichever comes first.

8. METHODS

8.1 Study parameters and study procedures

No blood samples will be collected.

8.2 Randomisation, blinding and treatment allocation

As per master protocol.

8.3 Study procedures

As per master protocol.

In addition, pregnancy should be ruled out by a negative urine pregnancy test for all women of child-bearing potential prior to randomisation.

8.4 Withdrawal of individual participant

As per master protocol.

8.5 Replacement of individual participants after withdrawal

As per master protocol.

8.6 Discontinuation of treatment of individual participants

As per master protocol.

In addition, a participant should discontinue treatment in case of:

- They experiences headache, nose bleed, or a burning sensation after use of the IMP. A physician should be consulted by the participant if symptoms persist.
- They become pregnant during IMP administration.

8.7 Premature termination of the study or arm

As per master protocol.

9. SAFETY REPORTING

9.1 Adverse events (AEs)

As per master protocol.

9.2 Serious adverse events (SAEs) will be recorded.

As per master protocol.

10. STATISTICAL ANALYSIS

For the primary analysis, Saline will be compared to Usual Care (see Appendix IIA: Usual Care). Superiority will be claimed if the posterior probability of superiority is greater than or equal to 0.988. Moreover, Saline will be used as a comparator for NONS.

Interim analyses

The first interim analysis will occur when 150 participants have been recruited to Saline (and 150 to Usual Care) and have had the opportunity to be followed for 28 days from randomisation. A second interim analysis will occur when 225 participants have been recruited to both arms. Recruitment to Saline will stop early for futility at either interim analysis if the posterior probability of superiority is less than 0.5, and for superiority if this probability is greater than 0.999.

11. ETHICAL CONSIDERATIONS

As per master protocol.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

As per master protocol.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

In theory, intranasal saline could physically rinse out nasal viruses, reducing viral load and attenuating the ensuing illness. Introduction of intranasal saline may additionally increase the viscosity and stability of mucous in the nasal passages, enhancing the barrier to viral penetration and subsequent infection of host cells [12, 13]. A direct antiviral effect of saline has also been described: *in vitro* work suggests that bathing DNA and RNA virus infected cells in increasing concentrations of sodium chloride solution leads to a dose-dependent inhibition of viral replication [14]. The inhibitory action is believed to be mediated by chloride ions entering host cells and forming hypochlorous acid (HOCl), which may in turn have a virucidal effect [14].

b. Previous exposure of human beings

These electrolytes are physiological. Previous RCT studies conducted with the saline solution comparator of NONS, as well as years of use in the community as over-the-counter (OTC) remedy, demonstrated a minimal risk.

Saline and was utilized in the following trials with no significant adverse safety signals;
1. India COVID-19 Phase 3 Treatment Randomized Clinical Trial – Q4, 2021-Q1, 2022 [15]
2. Bahrain COVID-19 Phase 3 Treatment Randomized Clinical Trial [16] (not published per IB)
3. Global COVID-19 Phase 3 Prevention Randomized Clinical Trial – Q1-3, 2022 [16] (not published, per IB)

c. Induction of the mechanism in animals and/or ex-vivo
These electrolytes are physiological; item(s) requested not applicable.

d. Selectivity of the mechanism
These electrolytes are physiological; item(s) requested not applicable.

e. Analysis of potential effect
These electrolytes are physiological; item(s) requested not applicable.

f. Pharmacokinetic considerations
These amounts of sodium and chloride are not expected to be of any harm. These electrolytes are physiological and administered at a physiological concentration. Plasma half-life is not applicable.

g. Predictability of effect
SARS-CoV-2 viral RNA concentration reduction (copies/mL) has been documented [15, 16].

h. Interaction with other products
For oral and parenteral medications, no drug-drug interactions are expected since the intranasally administered dose of saline is not expected to be systemically absorbed.

i. Managing of effects
These electrolytes are physiological; item(s) requested not applicable.

j. Study population
Pregnant/breastfeeding women and women of childbearing potential not adequately protected against pregnancy will be excluded.

13.2 Overall synthesis of the direct risks for the research subjects

These electrolytes are physiological. Previous RCTs conducted with saline as a control of NONS demonstrated minimal risk, as well as years of use in the community as OTC remedy. For this section reference is made to the latest Investigators Brochure.

References

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11. Šlapak, I., et al., *Efficacy of isotonic nasal wash (seawater) in the treatment and prevention of rhinitis in children.* Archives of otolaryngology–head & neck surgery, 2008. **134**(1): p. 67-74.
12. Edwards, D., et al., *A new natural defense against airborne pathogens.* QRB discovery, 2020. **1**.
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15. Tandon, M., et al., *SARS-CoV-2 accelerated clearance using a novel nitric oxide nasal spray (NONS) treatment: A randomized trial.* Lancet Reg Health Southeast Asia, 2022. **3**: p. 100036.
16. *NONS Investigators Brochure (IB), version 2.0 (update), November 2022.*