APPENDIX II INTERVENTION SPECIFIC APPENDICES (UK-SPECIFIC)

C. INVESTIGATIONAL PRODUCT: USUAL CARE + SALINE NASAL SPRAY

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 ECRAID-Prime's UK-specific master protocol.

3. STUDY DESIGN

3.1 Study Design

This IMP will be tested in a Phase IIb/III study.

3.2 Study Description

As per ECRAID-Prime's UK-specific master protocol, with the following changes for the Saline Nasal Spray evaluation:

- Start of IMP intake (Day 0) within 3 days since onset of symptoms.
- Swab at Day 0 and 4 by a healthcare professional (HCP) or self-taken.
- Study duration 3 months.

3.3 Schematic diagram of study design

As per ECRAID-Prime's UK-specific master protocol.

3.4 Duration of the study per participant

As per ECRAID-Prime's UK-specific master protocol. IMP self-administration for 7 days.

4. STUDY POPULATION

4.1 Population (base)

As per ECRAID-Prime's UK-specific master protocol.

4.2 Inclusion criteria

As per ECRAID-Prime's UK-specific master protocol. Additional inclusion criteria for Saline Nasal Spray arm (to be assessed by obtaining relevant information directly from the potential participant):

- Being able to start IMP intake within 3 days since onset of symptoms.
- For WOCBP*: have used and 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 ECRAID-Prime's UK-specific master protocol. Additional exclusion criteria for Saline Nasal Spray arm (to be assessed by obtaining relevant information directly from the potential participant):

- Known to be currently pregnant or breastfeeding.
- 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.

In case of recruitment by the central trial team, the central trial team clinician can decide to not enrol the potential participant if based on their clinical judgement the information provided by the potential participant is inconclusive for an eligibility decision.

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 either to the participating sites (GP practices, pharmacies) or to the accredited licensed facility at the PC-CTU, where the IMP can also be stored securely with restricted access, and be issued to participants directly by tracked courier service or by home delivery by the central trial team (e.g. research nurses).

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 [4]. 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] [5].

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 [6]. Their symptoms, elicited through questionnaires, were compared with historical controls of SARS-CoV-2 infected patients from February-March 2020 [6]. 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 [6]. 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) [7]. Participants grew a variety of viruses from their nasal passages, including rhinovirus, coronavirus, enterovirus and influenza virus [7].

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) [8]. 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 [8]. 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 [9]. 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 ($p<0.05$) [9].

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. 50 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.

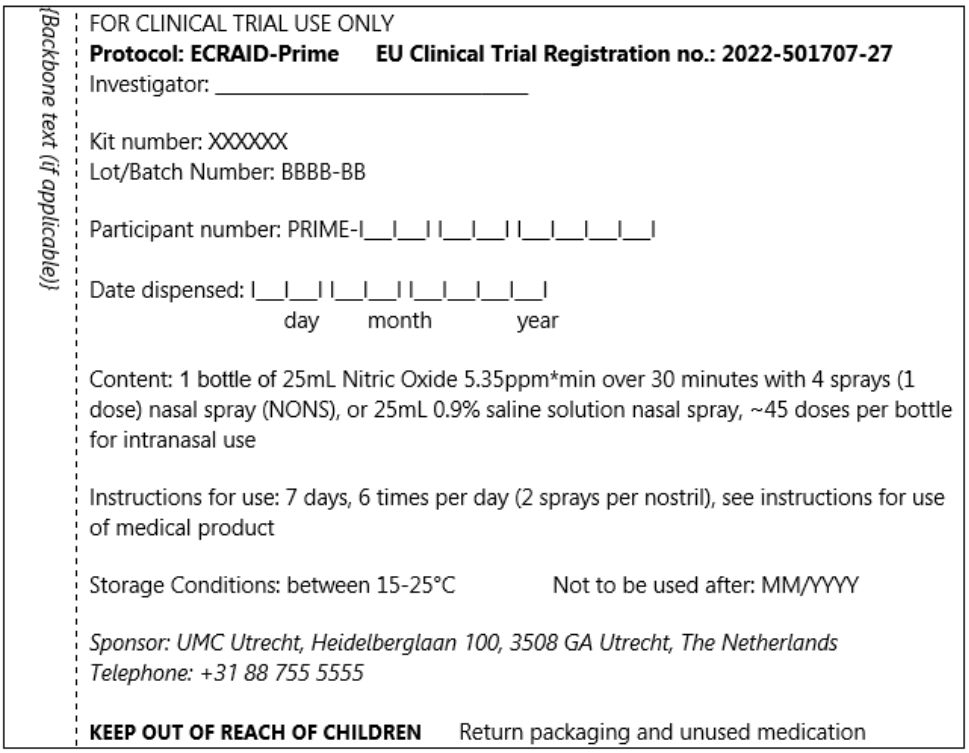
Saline dosing will follow the IMP schedule of previous randomized controlled trials (RCTs) with saline and nitric oxide nasal spray (NONS), i.e. India COVID-19 Phase 3 Treatment RCT, Bahrain COVID-19 Phase 3 Treatment RCT, and the revised Global COVID-19 Phase 3 Prevention RCT.

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 ECRAID-Prime's UK-specific 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 ECRAID-Prime's UK-specific master protocol and this ISA (section 4.2).

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/ endpoints

As per ECRAID-Prime's UK-specific master protocol.

8.2 Randomisation, blinding and treatment allocation

As per ECRAID-Prime's UK-specific master protocol, participants will be randomised using a rapid, secure, web-based randomisation system using non-deterministic minimisation, with a random element. The minimisation factors for the Saline Nasal Spray evaluation will be age (<55/≥55 years), time since onset of symptoms (≤2/3 days), and participating country.

8.3 Study procedures

As per ECRAID-Prime's UK-specific master protocol but with the following additions and changes:

1. Start of IMP intake within 3 days since onset of symptoms.
2. Baseline swab at Day 0 prior to starting IMP intake, and at Day 4 by a healthcare professional (HCP) or self-taken.
3. IP intake for 7 days.
4. Study duration 3 months.

8.4 Withdrawal of individual participant

As per ECRAID-Prime's UK-specific master protocol.

8.5 Replacement of individual participants after withdrawal

As per ECRAID-Prime's UK-specific master protocol.

8.6 Discontinuation of treatment of individual participants

As per ECRAID-Prime's UK-specific 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 ECRAID-Prime's UK-specific master protocol.

9. SAFETY REPORTING
9.1 Adverse events (AEs)
As per ECRAID-Prime's UK-specific master protocol. Adverse events will be collected for 2 weeks (i.e. twice as long as the IMP self-administration of 7 days).

9.2 Serious adverse events (SAEs) will be recorded.
As per ECRAID-Prime's UK-specific master protocol.

9.3 Pregnancy notification
As per ECRAID-Prime's UK-specific master protocol, the investigator has to notify to the Sponsor, immediately and no later than 48 hours after being made aware of a pregnancy, initiated during study participation, despite taken measures and precautions. Participants will be asked at Day 28, via their diary, whether they (WOCBP) have become pregnant. In situations where WOCBP have become pregnant within 28 days of study participation whilst being randomised to a (comparator)IP, unblinding will take place. If unblinding reveals that the participant did receive Saline Nasal Spray, pregnancy follow-up will not take place due to the well-known safety profile of Saline Nasal Spray when used during pregnancy and therefore follow-up of pregnancy is considered redundant.

10. STATISTICAL ANALYSIS
For the primary analysis, Saline Nasal Spray 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 Nasal Spray will be used as a comparator for NONS.

Interim analyses
The first interim analysis will occur when 150 participants have been recruited to Saline Nasal Spray (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 Saline Nasal Spray and 225 to Usual Care and have had the opportunity to be followed for 28 days from randomisation . Recruitment to Saline Nasal Spray 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 ECRAID-Prime's UK-specific master protocol.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION
As per ECRAID-Prime's UK-specific 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 [10, 11]. 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 [12]. 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 [12].

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 [13]
2. Bahrain COVID-19 Phase 3 Treatment Randomized Clinical Trial [14] (not published per IB)
3. Global COVID-19 Phase 3 Prevention Randomized Clinical Trial – Q1-3, 2022 [14] (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 [13, 14].

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

1. UK Health Security Agency. *COVID-19: epidemiology, virology and clinical features*. 2022 17th May 2022 [cited 2022 31st August 2022]; Available from: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features#:~:text=SARS%2DCoV%2D2%20is%20primarily,the%20eyes%2C%20nose%20or%20mouth.
2. Sungnak, W., et al., *SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes.* Nature medicine, 2020. **26**(5): p. 681-687.
3. Zhuang, M.W., et al., *Increasing host cellular receptor—angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection.* Journal of medical virology, 2020. **92**(11): p. 2693-2701.
4. Esther Jr, C.R., et al. *Pharmacokinetic-based failure of a detergent virucidal for severe acute respiratory syndrome–coronavirus-2 (SARS-CoV-2) nasal infections: A preclinical study and randomized controlled trial*. in *International forum of allergy & rhinology*. 2022. Wiley Online Library.
5. Zarabanda, D., et al., *The Effect of Povidone-Iodine Nasal Spray on Nasopharyngeal SARS-CoV-2 Viral Load: A Randomized Control Trial.* The Laryngoscope, 2021.
6. Spinato, G., et al., *The effect of isotonic saline nasal lavages in improving symptoms in SARS-CoV-2 infection: a case-control study.* Frontiers in Neurology, 2021: p. 2299.
7. Ramalingam, S., et al., *A pilot, open labelled, randomised controlled trial of hypertonic saline nasal irrigation and gargling for the common cold.* Scientific reports, 2019. **9**(1): p. 1-11.
8. Adam, P., M. Stiffman, and R.L. Blake Jr, *A clinical trial of hypertonic saline nasal spray in subjects with the common cold or rhinosinusitis.* Archives of family medicine, 1998. **7**(1): p. 39.
9. 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.
10. Edwards, D., et al., *A new natural defense against airborne pathogens.* QRB discovery, 2020. **1**.
11. Watanabe, W., et al., *Why inhaling salt water changes what we exhale.* Journal of colloid and interface science, 2007. **307**(1): p. 71-78.
12. Ramalingam, S., et al., *Antiviral innate immune response in non-myeloid cells is augmented by chloride ions via an increase in intracellular hypochlorous acid levels.* Scientific Reports, 2018. **8**(1): p. 1-11.
13. 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.
14. *NONS Investigators Brochure (IB), version 2.0 (update), November 2022.*