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PROTECT-CH TRIAL

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IMP-APPENDIX B to Protocol Version 1.0

**IMP: Niclosamide
IMP type: Post-exposure prophylaxis (PEP)**

IMP-Appendix agreed by:

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Table of Contents

1. Introduction and Rationale.....	3
2. Information on the Chemical and Pharmaceutical Properties	4
2.1 Active Substance	4
2.1.1 Drug Structure	4
2.1.2 Pharmacokinetic and Pharmacodynamic Properties	4
2.2 Manufacture and licensing status.....	5
2.2.2 Stability and Storage.....	5
3. Dosage and Administration	6
3.1 Prescription.....	6
3.2 Dosage	6
3.3 Administration/dispensing and return	6
3.4 Training.....	6
3.5 Length of treatment.....	6
3.6 Primary Endpoint	6
4. Additional Eligibility Criteria	7
4.1 Inclusion criteria.....	7
4.2 Exclusions and contraindications	7
5. Safety and Adverse Event Monitoring	7
5.1 Reference Safety Information	7
5.2 Adverse Events and Monitoring	7
5.2.1 Known side-effects.....	7
5.2.2 Reporting Adverse Events	7
6. Label.....	7
References	8



1. Introduction and Rationale

Niclosamide is a derivative of salicylic acid and is approved for treating tapeworm infestations and as a general piscicide in aquaculture. Although not licensed in the UK, the British National Formulary describes it as the most widely used drug for tapeworm infection.

Although the exact target(s) and mechanism(s) are known, niclosamide is a multimodal drug that inhibits or regulates multiple signalling pathways and biological processes via pleiotropic activities. These include acting as a protonophore and so uncoupling oxidative phosphorylation, reducing pro-inflammatory cytokines, inhibiting the STAT 3 signalling pathway and acidification of endosomes.[1] Recent studies have indicated that niclosamide may have broad clinical applications beyond the treatment of parasites. Experimentally, it exhibits activity against:

- Viruses - SARS-CoV-1/2,[2] MERS, rhinovirus, influenza, Chikungunya virus, Zika virus.
- Bacteria - *M. tuberculosis*, *P. aeruginosa*, methicillin-resistant *S. aureus*.
- Protect cells against bacterial toxins from - *B. anthracis* (anthrax) lethal toxin, *P. aeruginosa* exotoxin, *Corynebacterium* (diphtheria) toxin.
- Nematodes - *Caenorhabditis elegans*.

Niclosamide also has potential anti-cancer activity (adrenocortical carcinoma, breast, colorectal and cervical cancer, glioblastoma, hepatocellular carcinoma, head and neck and lung cancer, leukaemia, nasopharyngeal cancer, osteosarcoma, oral squamous cell carcinoma, ovarian, prostate, renal and thyroid cancer) and may modulate metabolic diseases (e.g. type II diabetes) and immune responses (e.g. rheumatoid arthritis).[3]

1.1 Anti-inflammatory effects

Niclosamide has been shown to have non-steroidal anti-inflammatory activity both experimentally and clinically:

- Reduce mucus production, bronchoconstriction and cytokine levels in a mouse model for asthma.
- Decrease clinical scores, joint swelling, inflammatory markers and pathological changes in arthritic rats.
- Prevents the formation of infected cellular syncytia in vitro at a concentration of 30nM probably through inhibition of TMEM16 activity.

1.2 Antiviral effects

In vitro laboratory studies, niclosamide has very potent SARS-CoV-2 activity in a variety of cell types:

- Vero FM cells - IC₅₀ 0.17 µM.
- Vero E6 cells - IC₅₀ 0.042-0.60 µM / 0.008-0.03 µg/mL; IC₉₀ 0.25-3.4 µM.[2, 4, 5]
- hACE2-A549 lung epithelial cells - IC₅₀ 0.15 µM; IC₉₀ 1.4 µM.[4]
- Calu-3 cells - IC₅₀ 0.084 µM.
- These potencies are greater than seen for chloroquine, lopinavir and remdesivir.
- Niclosamide inhibited SKP2 activity and enhances autophagy so reducing MERS-CoV replication.[6]

In vivo, intranasal niclosamide-lysozyme particles reduced death rates and viral loads in hACE2 transgenic mice infected intranasally with a lethal dose of SARS-CoV-2 (1E4 pfu).[5]

1.3 Phase I study

A randomized, placebo-controlled, double-blind, single-centre, dose-ascending Phase 1 trial assessed the safety of intranasal niclosamide (UNI91104, NCT04576312).[7] Healthy volunteers were randomly assigned to an ascending single dose in cohorts 1-4 and five doses over 2.5 days in cohort 5. Inclusion criteria included a minimum 80% of predicted lung function. Safety was



evaluated through adverse events (AEs) and pulmonary function tests including forced expiratory volume in one second (FEV1) and fractional exhaled nitric oxide (FeNO) tests. The primary endpoints were defined as the frequency of reported AEs and the change of safety variables relative to pre-dose. Data from all enrolled healthy volunteers receiving any amount of IMP was included in the primary analyses.[7]

Thirty-four healthy volunteers received UNI91104 and ten placebo.[7] No serious AEs or discontinuation were reported. Mild irritation in the upper respiratory tract following inhalation of niclosamide was reported as most frequent AE (45 events in 26 healthy volunteers, 59% of all healthy volunteers). Nasal application was well-tolerated. There was no evidence of difference in the change of mean levels of pulmonary function tests between active and placebo group across all cohorts. Five healthy volunteers (11.4%) (1 on placebo) had signs of increased transient FeNO and 4 on active (9.1%) experienced asymptomatic drops in FEV1, which resolved spontaneously or were reversible with a β 2-agonist. Niclosamide exhibited dose-proportional pharmacokinetics following inhalation and intranasal administration. The trial concluded that is well-tolerated in healthy volunteers and warrants further testing in patient trials.[7]

Phase I-II studies of niclosamide given only via the intranasal route have not been reported but the frequency of adverse events may be lower in view of the much lower administered doses of drug, i.e. 25.2 mg inhaled vs 2.5 mg intranasal.

1.4 Phase III trial

A trial of inhaled niclosamide is ongoing for the prevention of COVID-19 in patients with renal disease (haemodialysis, renal transplant, inflammatory renal diseases). 68 patients have been recruited of the intended 1500 (https://www.camcovidtrials.net/trials/view,protectv_50.htm).

2. Information on the Chemical and Pharmaceutical Properties

2.1 Active Substance

2.1.1 Drug Structure

5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxy-benzamide compd. with 2- aminoethanol

C₁₃H₈Cl₂N₂O₄

CAS: 50-65-7

ATC: P02DA01 and QP52AG03

Brand name: Not relevant

2.1.2 Pharmacokinetic and Pharmacodynamic Properties

Niclosamide anhydrous is a salicylanilide introduced as an oral anthelmintic in the early 1960s. Niclosamide ethanolamine is the slightly higher water-soluble salt of the compound niclosamide anhydrous. The major metabolites of niclosamide, identified in the Wistar rat after oral administration, are the O-glucuronide of niclosamide in bile (hydrolysed by β glucuronidase in the intestines), the 4'-nitro- reduced metabolite, (2',5-dichloro-4'-amino-salicylanilide) in the urine, and unchanged niclosamide in the faeces.



Niclosamide *in vitro* is a substrate and an inhibitor of CYP1A2 but plasma levels where CYP inhibition is seen *in vitro* are not expected to occur in humans administered intranasal niclosamide.

Since SARS-CoV-2 initially replicates predominantly in the nasal epithelium and *in vitro* data suggest that niclosamide inhibits SARS-CoV-2 replication and cellular penetration, nasal administration of niclosamide as a spray solution may be most effective as a post-exposure prophylactic for early stage infection when the viral load is a main issue.

The planned dose of 140 µL niclosamide as a nasal spray solution 1% per nostril twice daily, corresponds to 5.6 mg and 35.0 µg/cm² of niclosamide ethanolamine per day, assuming 100% deposition and a human nasal surface area of 160 cm².

With an assumption of 90 % protein binding and a 2 hour half-life, twice daily dosing would result in a free concentration of niclosamide that at peak concentration would be 857-fold higher than the IC₉₀ and after 6 half-lives (corresponding to the minimum exposure with the twice daily regimen) would be 13-fold higher than the IC₉₀. Based on these assumptions, the proposed highest feasible and well tolerated dose of inhaled niclosamide should lead to a continuous maintenance of a concentration at least 13-fold above the IC₉₀ in the nasal epithelium, which based on the available antiviral efficacy against SARS-CoV-2 would limit the ability the virus to establish in the nasal cavity and accordingly act as a treatment against infection. Assuming that 100% of the administered dose is absorbed and circulates, the maximal systemic exposure would still be approximately 43 times lower than that following 2 gm oral niclosamide.

2.2 Manufacture and licensing status

Niclosamide is not licensed for COVID-19 prophylaxis. The drug is currently approved and marketed for the oral treatment of tapeworm infections with administration of a single 2g regimen or 2g daily for 7 days in adults and children (>2 years of age). Oral niclosamide is currently marketed in several European countries, including Finland, France, Germany, Netherlands and Sweden under the trade names Yomesan, Kontal, and Tredemine.

UNION therapeutics have developed an intranasal application containing the active substance niclosamide for prophylaxis in patients at particularly high risk of COVID-19 and its complications.

UNION therapeutics will manufacture and distribute supplies of the product to a central pharmacy/manufacturing unit who will be responsible for the labelling and final packaging of the product into clinical trial supplies.

2.2.2 Stability and Storage

Stock niclosamide will be stored in the UK central pharmacy at 2°C - 8°C. Prescribed and dispensed stock to be distributed to care homes at ambient temperature.

Store below 25°C out of direct sunlight in accordance with the manufacturer's instructions.

Temperature monitoring will not be carried out for the purpose of the trial and storage should be managed and handled in accordance with the care homes own guidelines for the management of medicines. Individual prescriptions to be stored at room temperature for each resident as per usual care home practice.



3. Dosage and Administration

3.1 Prescription

Following randomisation, all participants within a care home randomised to receive niclosamide will be prescribed trial treatment by a PROTECT-CH doctor. Trial prescriptions will be sent directly to the central pharmacy. Upon receipt, the central pharmacy will apply approved trial-specific labelling (containing the participant's name) to the IMP and distribute trial medication directly to the care home who will be asked to confirm receipt.

Each participant will be prescribed 3 vials of Niclosamide Nasal Spray Solution 1% sufficient for the 6 week treatment duration. Each vial contains 8.5ml of the solution.

3.2 Dosage

Participants will administer niclosamide (1% in 20 ml) intranasal 140 µL spray into each nostril twice daily (equivalent to a total daily dose of 4.7 mg of niclosamide free acid).

3.3 Administration/dispensing and return

By registered nurses or care assistants trained to administer or assist in the administration of the intranasal spray device.

Any unused drug should be returned to a local pharmacy for destruction.

3.4 Training

Care home staff will be trained on the use of intranasal devices, and specific risks of airway obstruction from aspiration of loose objects (e.g. dentures). Prior to administration of IMP all staff involved in the administration of niclosamide will be required to complete a training module on the administration of the IMP.

3.5 Length of treatment

6 weeks.

3.6 Primary Endpoint

Day 60 from the point of randomisation. Care homes will be requested to enter primary endpoint data within 60+7 days.



4. Additional Eligibility Criteria

In addition to the inclusion/exclusion criteria detailed within the current version of the PROTECT-CH trial protocol, the following ADDITIONAL criteria will be used to assess a participant's eligibility to take niclosamide as an IMP in the trial:

4.1 Inclusion criteria

No additional criteria. Inclusion criteria as per protocol.

4.2 Exclusions and contraindications

1. Already taking, or definite need for, niclosamide.
2. Known allergy/hypersensitivity to niclosamide or any excipient.

5. Safety and Adverse Event Monitoring

5.1 Reference Safety Information

The Reference Safety Information for this IMP is the Investigators Brochure (IB) for UNI911 (Niclosamide nasal spray), Version 1.0, 26th August 2020 section 6.1 developed by UNION therapeutics. No SARS are considered expected for the purpose of expedited safety reporting.

5.2 Adverse Events and Monitoring

5.2.1 Known side-effects

For this trial the Reference Safety Information is: the current approved Union Therapeutics Investigator Brochure for UNI911 (Niclosamide nasal spray). No SARs are considered expected by the manufacturer for the purpose of expedited reporting of SUSARs and identification of SUSARs in the "Cumulative summary tabulation of serious adverse reactions" in the DSUR for the IMP.

5.2.2 Reporting Adverse Events

Serious Adverse Events (SAE), including Serious Adverse Reactions (SAR) and Suspected Unexpected SAR (SUSAR), will be recorded except where they constitute part of the primary outcome (all cause death, all cause hospitalisation and SARS-CoV-2 positivity). We will not collect adverse events (AEs) except those likely to occur with a nasal spray: nasal discomfort, headache, epistaxis.

Reporting will be via the on-line encrypted REDCap database.

6. Label

A trial-specific IMP label will be applied to the IMP by the central pharmacy prior to distribution to the care home.



References

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4. Wang, G., et al., *Fast and scalable lipid nanoparticle formulation of niclosamide (nano NCM) effectively inhibits SARS-CoV-2 replication in vitro*. bioRxiv, 2020.
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