UNION

THERAPEUTICS

PROTOCOL NUMBER: UNI911-101

STUDY DRUG: UNI911 INHALATION

Title:

Phase 1 Randomized, Double-Blind, Parallel Group, Placebo-Controlled Study to Assess the Safety of Ascending Doses of UNI911 INHALATION in Healthy Volunteers in Preparation for Evaluation in Adults with COVID-19

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1 CLINICAL PROTOCOL APPROVAL FORM

Protocol Title:	Phase 1 Randomized, Double-Blind, Parallel Group, Placebo-Controlled Study to Assess the Safety of Ascending Doses of UNI911 INHALATION in Healthy Volunteers in Preparation for Evaluation in Adults with COVID-19
Study No:	UNI911-101
Original Protocol	Version 3.0
Date:	28-Jun-2020

1.1 Sponsor Representative

This protocol is released and approved by UNION therapeutics A/S for the conduct of the clinical trial "*Phase 1 Randomized, Double-Blind, Parallel Group, Placebo-Controlled Study to Assess the Safety of Ascending Doses of UNI911 INHALATION in Healthy Volunteers in Preparation for Evaluation in Adults with COVID-19*". UNION therapeutics A/S will comply with all responsibilities of a sponsor as stipulated by Good Clinical Practice and by all applicable legislation and regulation.

Dr. Morten Sommer

Name | Role

Date | Signature

Dr. Philippe Andres

Name | Role

Date | Signature

1.2 National Coordinating Investigator

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the applicable legislation and regulation, with Good Clinical Practices as well with other applicable International Conference on Harmonization guidelines. Furthermore, I will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by UNION therapeutics A/S or specified designees. I will discuss the material with them to ensure that they are fully informed about UNION therapeutics A/S and the study.

_____
Investigator's Signature

Prof. Vibeke Backer, MD DMSci

_____
Printed Name

Rigshospitalet

_____
Name of Institution

Date: _____

Please submit an original copy of page 4 to UNION therapeutics and file a copy in the Investigator Site File.

1.3 Principal Investigator

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the applicable legislation and regulation, with Good Clinical Practices as well with other applicable International Conference on Harmonization guidelines. Furthermore, I will make a reasonable effort to complete the study within the time designated.

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_____
Investigator's Signature

Jesper Sonne, MD, DMSci

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Printed Name

DanTrials ApS

_____
Name of Institution

Date: _____

Please submit an original copy of page 5 to UNION therapeutics and file a copy in the Investigator Site File.

2 SYNOPSIS

Title:	Phase 1 Randomized, Double-Blind, Parallel Group, Placebo-Controlled Study to Assess the Safety of Ascending Doses of UNI911 INHALATION in Healthy Volunteers in Preparation for Evaluation in Adults with COVID-19
Protocol Number	UNI911-101
Investigators and Study Centers	National Coordinating Investigator (NCI): Professor Vibeke Backer, MD, DMSci Principal Investigator (PI): Jesper Sonne, MD, DMSci Study Center: DanTrials ApS, Bispebjerg og Frederiksberg Hospital Zelo Phase I Unit Indgang 11B, 1. sal, Nielsine Nielsens Vej 6B 2400 København NV, Denmark
Study Period	Estimated date first subject enrolled: June 2020 Estimated date last subject completed: August 2020
Objectives	This study is designed to assess the safety of UNI911 INHALATION in healthy volunteers (HVs) following both single ascending dose and multiple dosing as preparation for a study to assess safety and efficacy in adults with COVID-19. **Primary Objective:** Assess safety of UNI911 INHALATION in healthy volunteers
Rationale	UNI911 is comprised of the of the compound niclosamide (5-chloro-N-[2-chloro-4-nitrophenyl]-2-hydroxybenzamide), which is a salicylanilide introduced in early 1960s and primarily used as an anthelmintic drug [1, 2], both in human medicine (adults and children) as well as in veterinary medicine. The drug has been used extensively for indications outside parasitic infections and is listed in the WHO Model List of Essential Medicines [3]. Niclocide®, (niclosamide), an anthelmintic, was approved in the U.S. as a 500 mg chewable oral tablet (Niclocide; NDA 018669, Bayer Pharms; May 14, 1982). Niclocide has been indicated for the treatment of tapeworm infections by *Taenia saginata* (beef tapeworm), *Diphyllobothrium latum* (fish tapeworm), and *Hymenolepis nana* (dwarf tapeworm). For the treatment of tapeworms in adults, Niclocide was administered as a single 2 g dose (4 tablets) (*Taenia saginata* or *Diphyllobothrium latum*) or 2 g (4 tablets) QD for 7 days (*Hymenolepis nana*). Niclocide® was discontinued in 1996; the NDA holder notified the Agency that the drug product was no longer marketed and requested that the approval of the application be withdrawn (Federal Register Vol. 61, No.192 pg. 51457). Oral niclosamide is currently marketed in several European countries, including Finland, France, Germany, Netherlands, and Sweden under the trade names Yomesan®, Kontal® and Tredemine®. It is a 500 mg tablet and in human medicines, single oral doses of 0.5, 1 and 2 g niclosamide are recommended for children under 2 years, between 2 and 6 years and for children older than 6 years and adults, respectively.

When treating human volunteers with a single oral dose of 2,000 mg niclosamide, maximal serum concentration of niclosamide was equivalent to 0.2-6 μg/mL (0.61 – 18.3 μM). The wide concentration range was caused by the intra-individual absorption differences. Niclosamide is only partially absorbed from the intestinal tract (with bioavailability reported between 10 and 20%), and the absorbed part is rapidly metabolized by the liver and eliminated by the kidneys. These characteristics hamper its extensive clinical development as a systemic agent (Yomesan® SmPC; [4]).

Coronaviruses are a group of enveloped and non-segmented positive-sense RNA viruses with very large genome size ranging from approximately 27 - 34 kb. Infections with human strains HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 usually cause mild, self-limiting respiratory infections, such as the common cold [5, 6]. Nevertheless, in the past 17 years, three betacoronaviruses, SARS-CoV, MERS-CoV and this year's SARS-CoV-2, have caused severe human disease epidemics associated with high morbidity and mortality. The recent outbreak of coronavirus disease 2019 (COVID-19) first detected in Wuhan, China, was caused by a novel beta coronavirus, which was named as SARS-CoV-2 (also known as 2019-nCoV) by the *International Committee on Taxonomy of Viruses*.

The lack of effective treatment for coronavirus infections poses a great challenge to clinical management and highlights the urgent need for new drug discovery.

Notably, niclosamide is a potent inhibitor of SARS-CoV and SARS-CoV2 replication. First, Wu *et al.* found that niclosamide was able to inhibit SARS-CoV replication *in vitro* and totally abolished viral antigen synthesis at a concentration of 1.56 μM, after screening a small marketed drug library [7]. Niclosamide suppressed cytopathic effect of SARS-CoV at concentration as low as 1 μM and inhibited SARS-CoV replication with an IC50 value of less than 0.1 μM in Vero E6 cells [8]. Very recently, niclosamide was found to be a very potent inhibitor of SARS-CoV2 with an IC50 of 280 nM [9].

In addition to this potent antiviral activity against SARS-CoV-2, niclosamide holds two other important properties: (1) anti-inflammatory activity *in vivo* in lungs accompanied by mucus reduction and bronchodilation [10].

These findings indicate that niclosamide may be repurposed as a treatment of COVID-19 that inhibits viral replication, limits inflammation and, secondarily, prevents superinfection.

Considering the unfavorable PK profile of niclosamide delivered orally, UNION therapeutics initiated a development program on topical forms of the compound in dermatology. A Phase 1 study evaluated the irritation potential of topically applied ATx201 OINTMENT 2% and 4%, along with vehicle and positive (sodium lauryl sulfate 0.05%) and negative (distilled water for injection) controls, in healthy subjects on intact and abraded skin (via tape stripping). Thirty-six healthy adult subjects received all the test articles applied to the upper back daily for 21 days. The Cumulative Irritation Score for the two concentrations, on abraded and intact skin was lower than the negative control, confirming the good local tolerance of topical niclosamide.

A study of PK under maximal use conditions in 17 adult subjects recently confirmed the good safety profile and very low systemic levels of niclosamide 7% applied topically in atopic dermatitis. A Phase 2b study comparing two

	concentrations of topical niclosamide (4% and 7%) to vehicle, is currently being conducted in 210 subjects with mild to moderate atopic dermatitis. A similar approach is proposed for topical applications to the respiratory system *via* inhalation. Given the urgent need for new therapies, the promising pharmacological activities and favorable local and systemic safety profile of niclosamide, UNION therapeutics is now developing UNI911 as an inhalable formulation for the treatment of COVID-19 caused by SARS-CoV-2.
Methodology/ Study Design	This is an ascending dose scaling study in adult HVs to assess the safety of four increasing doses of UNI911 INHALATION. 44 healthy volunteers will be enrolled in five sequential cohorts: • Cohort 1: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (4 mL, 0.1%, equaling 3.4 mg niclosamide) and nasal spray (2 x 150 µL, 0.1%, i.e. once per nostril, totaling 0.25 mg niclosamide) and 2 to receive placebo. • Cohort 2: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (1 mL, 1%, equaling 8.4 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo. • Cohort 3: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (3 mL, 1%, equaling 25.2 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo. • Cohort 4: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (6 mL, 1%, equaling 50.4 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo. • Cohort 5: 8 healthy volunteers, 6 to receive five doses of UNI911 INHALATION (6 mL, 1%, equaling 50.4 mg niclosamide per dosing and 252 mg in total) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide per dosing and 12.6 mg in total) dosed BID for 2.5 days and 2 to receive placebo. Screening and enrollment for the cohorts will be initiated in parallel, while dosing will be done sequentially. Dosing will begin with cohort 1. Once data from a minimum of 8 subjects (i.e., at least 6 subjects on active treatment) from cohort 1 is available, the safety parameters will be assessed by a Safety Monitoring Committee (SMC) before initiating dosing of subjects in cohort 2. Similarly, the SMC will review the data from all exposed subjects and a minimum of 8 subjects in cohorts 2, 3 and 4 before opening for dosing in the subsequent cohorts (3, 4 and 5 respectively). Based on the results of this study, the SMC will make a recommendation concerning the dosing in a subsequent Phase 2 study in patients with COVID19 to be initiated immediately after UNI911-101. For the four first cohorts, one sentinel subject will be dosed with UNI911 INHALATION (open-label) the first day and followed for 24 hours, while admitted at the clinic to confirm the safety of the new dose before dosing the remaining subjects in the cohort. If safety concerns are observed, the SMC will be involved to adjudicate;

	if no safety concerns are observed or the SMC judges it is safe to continue dosing, the remaining 8 subjects in each cohort will be randomized and dosed (double-blinded) with an interval of at least one hour. Once safety and PK data are available from all cohorts, the SMC will assess the safety parameters and review the PK data to confirm safety of the four doses. Based on this, the SMC will recommend a dose for administration in COVID-19 patients in the next phase of development. Screening may be performed up to 21 days before initiation of study treatment[1]. For subjects to qualify for enrollment in the study, they cannot be smokers, should be in good general health and have a normal medical history excluding any chronic disease as per the investigator's judgment, as well as minimum 80% of predicted lung function, including Forced Expiratory Volume in 1 second (FEV1) after beta2-agonist, Total Lung Capacity (TLC), Carbon Monoxide Diffusion capacity (DCO), and a cardiopulmonary exercise testing (CPET) with pulse oximetry. Finally, vital signs, ECG and chest X-ray must not be clinically significantly abnormal. (See Exclusion criteria for full details.) Investigational Product (IP) will be a single ascending dose, multiple doses (BID), or placebo administered by qualified study staff, after which the subject will be followed for 24 hours in the clinic and return for a final check 48 hours after dosing. A general physical examination, serum chemistry and hematology sampling as well as urinalysis will be performed at screening, 24, and 48 hours after last dose. If the first screening visit is conducted more than 3 days before first dosing, the subject must come to the clinic 1-3 days before dosing for an oropharyngeal swab (to confirm no infection with SARS-CoV2) and sampling for serum chemistry, hematology, and urinalysis. In terms of respiratory function, safety will be assessed on the basis of spirometry (vital capacity and FEV1) , pulse oximetry and diffusion capacity : • Vital capacity and FEV1 + Pulse oximetry: o Cohort 1-4: pre-dose, 1, 3, 6, 12, and 24 hours after dosing. o Cohort 5: ▪ Day 0 and Day 1: pre-dose, 1, 3 hours after morning and evening dosing ▪ Day 2: pre-dose, 1, 3, 6, 12, 24 hours after morning dosing o • DCO: o Cohort 1-4 : Predose, 1 and 3 hours after dosing o Cohort 5: not applicable (will be done at screening and on Day 4, see below) In addition, FEV1 (including reversibility), TLC, DCO, and FeNO will also be measured and a CPET with pulse oximetry will be conducted during the

1 An oropharyngeal swab will be collected between 1-3 days before dosing to confirm that HV subjects are not infected with COVID-19

	screening period (between ICF signing and dosing) and on Day 2 after dosing[1] for cohort 1-4 and on Day 4 for cohort 5. ECGs will be captured at • Cohort 1-4: o Screening o Day 0: pre-dose, 3, 6, and 24 hours after dosing, • Cohort 5: o Screening o Day 0: pre-dose, 3 hours after the morning dose o Day 2: pre-dose, 3, 6, and 24 hours after dosing. Vital signs (systemic blood pressure, pulse, respiratory rate (RR), and body temperature) will be measured at • Cohort 1-4: screening, pre-dose, 1, 3, 6, 12, 24, and 48 hours post dose. • Cohort 5: o Screening o Day 0 and Day 1: pre-dose, 1, 3 hours after morning and evening dosing o Day 2: pre-dose ,1, 3, 6, 12, 24 and 48 hours after morning dosing AEs will be collected from the time of first dose and through-out the study period. Finally, an oropharyngeal swab for detection of viruses and bacteria will be taken pre-dose and 48 hours after dosing for *post hoc* exploratory analysis of potential changes in the microbiome. Blood samples for PK analysis will be collected pre-dose, ½, 1, 1½, 2, 3, 6, 12, and 24 hours after dosing in cohorts 1 – 4. In cohort 5, samples will be taken in the morning on Day 0 at pre-dose and +1, +3 and +6 hours after dosing, and in the evening: Pre-dose and +1 and +3 hours after dosing. No PK sampling will be done on Day 1. On Day 2 Blood samples for PK analysis will be collected pre-dose, ½, 1, 1½, 2, 3, 6, 12, 24, 48 hours after dosing.
Number of Subjects	44subjects will be sequentially enrolled to receive dosage of UNI911 INHALATION and nasal spray or placebo. 4 sentinel subjects (one per cohort 1, 2 , 3 and 4) will be treated in an open-label manner to confirm the safety of each dose sequentially. The remaining 40 subjects will be randomly assigned (3:1) to either active or placebo.

1 The day of dosing is designated as Day 0

Inclusion Criteria	Subjects are only eligible if they fulfill all criteria for inclusion: (1) Signed Informed Consent Form (ICF) (2) Male or non-pregnant and non-lactating female who is abstinent or agrees to use effective contraceptive methods throughout the course of the study. Females must have a negative urine beta-human chorionic gonadotropin hormone (hCG) pregnancy test prior to dosing. (Women who are postmenopausal[1] or who had tubal ligation/hysterectomy do not need to have a pregnancy test done and do not need to agree to use contraception.) Acceptable birth control methods are the following: • Intrauterine device in place for at least 3 months • Stable hormonal contraceptive for at least 3 months prior to dosing and continuing through study completion • Surgically sterilized partner (3) ECG without clinically significant abnormalities (including QTcF < 450 ms) (4) Age ≥ 18 and < 65 years at the time of signing ICF (5) Normally active and in good health by medical history and physical examination (6) Minimum 80% of predicted lung function, including FEV1 after beta2-agonist, TLC, DCO, and CPET with pulse oximetry (7) Chest X-ray without clinically significant abnormalities
Exclusion Criteria	Subjects who meet any of the following criteria are not eligible to participate in this study: (1) Enrollment in an UNI911 study in the previous 6 months (2) Clinically significant allergy (as judged by the investigator) or history of significant adverse reaction to niclosamide or related compounds, to any of the excipients used. (3) Underlying condition that may interfere with inhalation of the IP (4) Current acute or chronic condition (incl. COPD, asthma, or other severe respiratory disease, CV disease, diabetes mellitus, obesity, malignant and autoimmune diseases) unless considered clinically irrelevant and stable by the investigator (5) Renal impairment (eGFR[2] < 60 mL/min/1.73m^2) or hepatic impairment (as judged by the investigator) (6) The presence of a condition which renders the subject "vulnerable " as defined by GCP or of a condition the investigator believes would interfere with the ability to provide informed consent, or comply with study procedures/instructions, or that might confound the interpretation of the study results or put the subject at undue risk (7) Smoke or regular use of any form of nicotine product incl. e-cigarette, snuff,

1 Menopause is defined as the time when there have been no menstrual periods for 12 consecutive months and no other biological or physiological cause can be identified.

2 eGFR estimated by CPK-EPI

	chewing tobacco, nicotine gum, etc., during the study period and for the previous 6 months (8) Known difficulty undergoing venipuncture or poor venous access (9) Whole blood donation or loss (> 400 mL) within 90 days before the dosing of IP (10) History of any malignancy except subjects with adequately treated basal cell / squamous carcinomas of the skin (11) Consumed alcohol in the 24 hours prior to dosing *Prior or concomitant therapy* (12) Any systemic and inhaled therapies 5 half-lives prior to dosing (hormone replacement therapy for postmenopausal women and hormonal anticonception are allowed). (13) Participation in any clinical trial 90 days prior to dosing of UNI911 INHALATION or placebo
Test Products, Dose, Mode of Administration, and Duration of Treatment	Qualified staff will administer UNI911 INHALATION and nasal spray • 0.1% or placebo once to healthy volunteers in cohort 1 • 1.0%, or placebo once to healthy volunteers in cohort 2, 3 and 4, • 1. 0 % or placebo twice daily for 2.5 days to healthy volunteers in cohort 5. Inhalation is performed by using an EN 13544-1 certified nebulizer and administration via the intranasal mucosa by using
Duration of Study	Subject participation in the study (excluding the screening period) is approximately 3 days in cohorts 1-4 and 5 days in cohort 5, not including potential follow-up of ongoing (S)AEs or pregnancies.
Safety Variables	In addition to AEs/SAEs collection throughout the study duration, general safety will be assessed via clinical examination, vital sign assessments, ECGs, and laboratory analysis (serum chemistry, hematology, and urinalysis). Pulmonary function will be particularly followed with specific tests including measurements of vital capacity, expiratory volume (FEV1), static volume (TLC), diffusion capacity (DCO), exhaled nitric oxide (FeNO), resting pulse oximetry as well as a CPET.
PK Variables	Cohort 1, 2 , 3, and 4: Blood samples for PK analysis will be collected pre-dose, ½, 1, 1½, 2, 3, 6, 12, and 24 hours after dosing Cohort 5: Blood samples for PK analysis will be collected on Day 0 in the morning at pre-dose, 1, 3, and 6 hours after dosing and in the evening at pre-dose, 1 and 3 hours after dosing. No PK sampling will be done on Day 1. On Day 2, blood samples for PK analysis will be collected at pre-dose, ½, 1, 1½, 2, 3, 6, 12, 24, and 48 hours after dosing.

Endpoints and Criteria for Evaluation	**Primary endpoints** o AE frequency in each cohort and treatment group o Change from baseline for all safety variables measured and frequency of out of range values **Exploratory end points (to be analyzed post hoc)** o Oropharyngeal microbiome changes **Key PK Parameters** o Maximum concentration of active drug molecules in blood (C_{max}) o Time to reach maximum level (T_{max}) o Area Under the Curve of drug level in blood versus time (AUC) o Half life
Sample Size	The study is not powered for inferential statistics. 44 healthy volunteers will be enrolled such that 7 (6 blinded and 1 open label) subjects are treated with a single ascending dose plus nasal spray of UNI911 INHALATION 0.1% in cohorts 1 and UNI911 INHALATION 1% in cohorts 2 , 3 and 4. In cohort 5, 6 blinded subjects are treated with multiple doses plus nasal spray of UNI911 INHALATION 1%. In each of the five cohorts 2 subjects are treated with placebo (blinded). This sample size is considered sufficient to meet the study objectives but is not based on statistical power considerations.
Principal Statistical Methods	Details of the statistical analysis will be provided in a separate Statistical Analysis Plan (SAP). Continuous variables will be summarized by cohort at each visit in tables and will include the number of subjects, mean, standard deviation, median, minimum, and maximum. Categorical variables will be presented by cohort at each visit in tables as frequencies and percentages. In general, for each parameter, both the raw value at each visit and its change or percent change from baseline when appropriate will be presented. Observed cases (i.e., non-missing) will be presented. No imputation for missing data will be made. **Analysis sets and missing data imputations** The Pharmacokinetic (PK) Analysis Set will include data from subjects who were treated and had no missing data affecting the PK assessment. The Safety Analysis Set includes data from all enrolled subjects receiving any amount of IP. **Descriptive statistics** Demographical data will be presented in tables per phase and for the full study population. **Safety** All safety analyses will be conducted using the Safety Analysis Set. Adverse event data will be presented and tabulated according to MedDRA classification.

	Reported AEs will be summarized by the number of subjects reporting the events and by System Organ Class (SOC) and Preferred Term (PT), severity and seriousness; and SOC, PT, and relationship to IP. The outcome of AEs will be reported. The lung function measurements (TLC, DCO, FEV1, FeNO, CPET) and blood oxygen saturation will be presented in tables and graphically per cohort. Laboratory (serum chemistry, hematology, and urinalysis) parameters and vital signs will be tabulated by visit using descriptive statistics. The value at each visit as well as the change from baseline will be presented. ECG data and the findings of the physical examination will be reported as listings with abnormal and clinically relevant values identified. Numerical data are summarized as applicable per cohort. **Pharmacokinetic analysis** All pharmacokinetic analyses will be conducted using the PK Analysis Set. Non-compartmental pharmacokinetic parameters will be reported as geometric mean, %CV, minimum and maximum including: • C_{max} • T_{max} • AUC • Half-life Finally, the concentration will be plotted vs. nominal time for the PK profile both for individual subjects and as mean concentration per cohort.

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4 ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
ADL	Activity of Daily Life
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical Classification System
AUC	Area under Curve
BID	*bis in die*, two times/d, with a minimum time span of 8 hours between subsequent administrations
BioFire®	BioFire® Filmarray® -Respiratory Panel
BUN	Blood Urea Nitrogen
CDF	Cumulative Distribution Function
C_{max}	Maximum Concentration of Active Drug Molecules in Blood
CMCO	Carbon Monoxide Diffusing Capacity
CNS	Concentrate for Nebulizer Solution
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Infectious disease caused by SARS-CoV-2
COX	Cyclooxygenase
CPE	Cytopathic Effect
CPET	Cardiopulmonary Exercice Testing
CRP	C-Reactive Protein
CRO	Clinical Research Organization
CS	Clinically Significant
CT	Computer Tomography
CTCAE	Common Terminology Criteria for AEs
CV	Cardiovascular
D	Day
DCO	Carbon Monoxide Diffusion Capacity
DM	Data Manager
DMP	Data Management Plan
DVP	Data Validation Plan
ECG	Electro Cardiography
EC_{50}	Half Maximal Effective Concentration
CRF	Case Report Form

Abbreviation	Definition
F	Availability
FDA	US Food and Drug Administration
FeNO	Fraction of Expiratory Nitric Oxide
FEV1	Forced Expiratory Volume in 1 s
GCP	ICH E6 Good Clinical Practice [11]
G-CSF	Granulocyte-Colony Stimulating Factor
GFR	Globular Filtration Rate
HbA1c	Glycated hemoglobin
hCG	β-Human Chorionic Gonadotropin Hormone
HDL	High Density Lipoprotein
HV	Healthy Volunteer
IB	Investigator's Brochure
IC_{50}	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
ID	Identification Number
IEC	Independent Ethics Committee
IFNγ	Interferon γ
IL1B	Interleukin 1B
IP	Investigational Product
IP10	C-X-C Motif Chemokine 10, also known as γ-IP10 or 10 kDa interferon gamma-induced protein
ITT	Intent to Treat
kb	10^3 base pairs
LDL	Low Density Lipoprotein
LOCF	Last Observation Carried Forward
Max	Maximum
MCP1	Monocyte Chemotactic Protein 1
MedDRA	Medical Dictionary for Regulatory Activities
MERS-CoV	Middle East Respiratory Syndrome Coronavirus, a Betacoronavirus for the Family Coronaviridæ (Nidovirales, Riboviria)
Min	Minimum
MIP1A	Macrophage Inflammatory Protein 1α, also known as CCL3
MRSA	Methicillin-Resistant *Staphylococcus aureus*

Abbreviation	Definition
NCI	National Coordinating Investigator
NCS	Not Clinically Significant
NDA	New Drug Application
O_2	Oxygen
PD	Protocol Deviation
PI	Principal Investigator
PK	Pharmacokinetics
EN 13544-1	DIN EN 13544-1:2009-12: Respiratory Therapy Equipment - Part 1: Nebulizing Systems and their Components. Dec 2009
PT	Preferred Term in the terminology of MedDRA
QD	*quaque die*, one/d
QID	*quater in die*, four times/d
QTcF	QT Interval after Friedrich
RR	Respiratory Rate
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus, a Betacoronavirus for the Family Coronaviridæ (Nidovirales, Riboviria)
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2, a Betacoronavirus for the Family Coronaviridæ (Nidovirales, Riboviria), also known as 2019-nCoV
SD	Standard Deviation
SMC	Safety Monitoring Committee
SmPC	Summary of Product Characteristics
SoC	Standard of Care
SOC	System Organ Class in the terminology of MedDRA
SOFA	Sequential Organ Failure Assessment. This study uses the 0 – 24 scale [12]
SUSARs	Suspected Unexpected Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TLC	Total Lung Capacity
T_{Max}	Time to Reach Maximum Level
TNFα	Tumor Necrosis Factor Alpha
UNI911	UNI911 for inhalation, the IP of this study

Abbreviation	Definition
Well-Baby Visit	An outpatient visit at a pediatrician in order to monitor the status and the development of a presumably healthy child
WHO	World Health Organization
WoCBP	Women of Childbearing Potential
2019-nCoV	Alternative name for SARS-Co-V 2
21CFR Part 11	Code 21 of the (US) federal regulation, part 11

5 INTRODUCTION AND RATIONALE

5.1 Introduction

This study tests the safety of UNI911 INHALATION, the compound niclosamide (5-chloro-N-[2-chloro-4-nitrophenyl]-2-hydroxybenzamide) in healthy volunteers (HVs) with the final aim of using this substance for the treatment of COVID-19, caused by the coronavirus SARS-CoV-2.

Coronaviruses are a group of enveloped and non-segmented positive-sense RNA viruses (Riboviria) with very large genome size ranging from approximately 27 - 34 kb. Infections with human strains HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 usually cause mild, self-limiting respiratory infections, such as the common cold [5, 6]. Nevertheless, in the past 17 years, three beta coronaviruses, SARS-CoV, MERS-CoV and this year's SARS-CoV-2, have caused severe human disease pandemics associated with high morbidity and mortality. The recent outbreak of coronavirus disease 2019 (COVID-19) first detected in Wuhan, China, was caused by a novel betacoronavirus, which was named as SARS-CoV-2 (also known as 2019-nCoV) by the International Committee on Taxonomy of Viruses [13–15]. Now considered a pandemic by the WHO [16], COVID-19 affects (by 10-May-2020) 3,917,000 people in 215 countries and caused 274,361 deaths [16]. In Denmark, 10,319 cases and 526 deaths were observed until 10-May-2020 [16]. In addition to increased morbidity [17, 18], the pandemic caused catastrophic socioeconomic effects in Denmark and worldwide [19].

The lack of effective treatment for coronavirus infections poses a great challenge to clinical management and highlights the urgent need for new drug discovery [20]. Niclosamide is a potent inhibitor of SARS-CoV replication *in vitro* [7]. Recently, this anti-viral activity was confirmed for SARS-CoV-2 [9, 21]. The potent anti-viral activity of niclosamide in

combination with its favorable safety profile and other pharmacological activities renders niclosamide a promising candidate for the treatment of COVID-19.

5.2 Rationale

Niclosamide (5-chloro-N-[2-chloro-4-nitrophenyl]-2-hydroxybenzamide) is a salicyl-anilide originally developed as an anthelminthic drug in the early 1960s, with a well-established safety profile due to decades of safe oral use. It is listed in the WHO Model List of Essential Medicines [3, 22]. The underlying mechanism involves uncoupling of oxidative phosphorylation, and modulation of Wnt/β-catenin, mTORC1, STAT3, NF-κB and Notch signaling pathways.

Niclocide® (niclosamide), an anthelmintic, was approved in the U.S. as a 500 mg chewable oral tablet (Niclocide; NDA 018669, Bayer Pharms; May 14, 1982 [23]). Niclocide® has been indicated for the treatment of tapeworm infestations by *Taenia saginata* (beef tapeworm), *Diphyllobothrium latum* (fish tapeworm), and *Hymenolepis nana* (dwarf tapeworm). For the treatment of tapeworms in adults, Niclocide® was administered as a single 2 g dose (4 tablets) (*Taenia saginata* or *Diphyllobothrium latum*) or 2 g (4 tablets) QD for 7 days (*Hymenolepis nana*) [1, 2]. Niclocide® was discontinued in 1996; the NDA holder notified the Agency that the drug product was no longer marketed and requested that the approval of the application be withdrawn (Federal Register Vol. 61, No.192 pg. 51457). Oral niclosamide is currently marketed in several European countries, including Finland, France, Germany, Netherlands, and Sweden under the trade names Yomesan®, Kontal® and Tredemine® as a 500 mg tablet. In human medicines, single oral doses of 0.5, 1 and 2 g niclosamide are recommended for children under 2 years, between 2 and 6 years and for children older than 6 years and adults, respectively. Additional information can be found in the UNI911 COVID Investigator's Brochure.

When treating human volunteers each with a single oral dose of 2,000 mg niclosamide, maximal serum concentration of niclosamide was equivalent to 0.2-6 µg/mL (0.61 – 18.3 µM). The wide concentration range was caused by the intra-individual absorption differences. Niclosamide is only partially absorbed from intestinal tract (with bioavailability reported between 10 and 20%), and the absorbed part is rapidly eliminated by the kidneys. These characteristics hamper its extensive clinical development as a systemic agent (Yomesan® SmPC; [4]).

Recently, niclosamide was found to exert positive effects on several diverse diseases outside parasitic infestations [24]. These findings indicate additional potential indications for the substance, including but not restricted to:

- **Anti-inflammatory activity** *in vivo* in lungs accompanied by mucus reduction and bronchodilation [10].

- **Antiviral activity**: Niclosamide is a potent inhibitor of SARS-CoV and SARS-CoV2 replication (Figure 1). First Wu *et al.* found that niclosamide was able to inhibit SARS-CoV replication *in vitro* and totally abolished viral antigen synthesis at a concentration of 1.56 µM after screening a small marketed drug library [7]. Niclosamide suppressed cytopathic effect of SARS-CoV at concentration as low as 1 µM and inhibited SARS-CoV replication with an IC50 value of less than 0.1 µM in Vero E6 cells [8]. Very recently, niclosamide was found to be a very potent inhibitor of SARS-CoV2 with an IC50 of 280 nM [9]. These tests show that niclosamide exhibits a more pronounced antiviral activity than remdesivir and chloroquine. Unlike chloroquine, niclosamide has no cytotoxic effects in the concentration range tested.

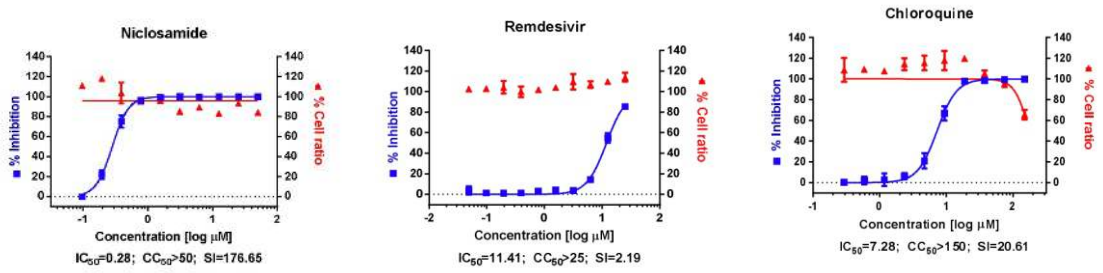

Figure 1: Antiviral (blue) and cytotoxic (red) effects of niclosamide in comparison to two other candidates, remdesivir and chloroquine [9].

These findings indicate that niclosamide may be repurposed with the therapeutic potential applications to combat COVID-19 by inhibiting viral replication, limiting inflammation and, secondarily, preventing superinfection.

Considering low bioavailability of niclosamide administered orally, UNION therapeutics initiated a development program on topical forms of the compound. To date, more than 500 subjects aged down to 9 months have been treated with topical niclosamide with no Severe Adverse Events or other significant safety concern. Most recently a study in 17 adult subjects confirmed the good safety profile and very low systemic levels of niclosamide 7% applied topically in atopic dermatitis patients. A Phase 2b study comparing two concentrations of topical niclosamide (4% and 7%) to vehicle, is currently conducted in 210 subjects with mild to moderate atopic dermatitis [25, 26]. A similar approach is proposed for topical applications to the respiratory system via inhalation.

Given the urgent need for new therapies, the promising pharmacological activities and favorable safety profile of niclosamide, UNION therapeutics is now developing UNI911 INHALATION as inhalable formulation for the treatment of COVID-19 caused by SARS-CoV-2.

This product will be delivered to the lung thanks to a marketed nebulization device, the rapid Nebuliser System, developed by the leading company . While nebulization ensures proper delivery of the drug to the airways and alveolar sacs, this procedure does not allow deposition of the drug to the nasal cavity. Given that this anatomical zone is another major area for viral replication, nebulization procedure will be associated with the spraying of the same formulation directly into each nostril. This will be achieved thanks to a specific needle free device designed and marketed for optimal atomization of nasal medications (Intranasal Mucosal Atomization Device from Teleflex). Both of these devices are CE marked and their description can be found in the protocol appendices.

5.3 Justification of Dose

This study serves to identify the maximal tolerated dose of UNI911 INHALATION in five cohorts of healthy volunteers, exposed to 0.1% and 1.0% niclosamide solution in nebulized form or as a nasal spray corresponding to a total quantity of niclosamide administered ranging from 3.65 mg in one dose in cohort 1 to 264.6 mg in five doses in cohort 5 (52.9 mg per dose).

Niclosamide (Yomesan®) is approved in several European member states as an oral treatment for tapeworm infections. There is no prior clinical experience with treatment of niclosamide by inhalation. Therefore, the dosing regimen in the proposed study considers published nonclinical data in rats as well as clinical data after oral administration

With regard to potential systemic exposure after inhalation, the proposed initial dose for inhalation (4 mL at a concentration of 0.1%, once daily) corresponds to a total dose of 3.4 mg of niclosamide. Even in a highly conservative approach where 100% of the administered dose would be assumed to permeate and reach blood circulation (assumingly 80% reaches the respiratory tract and the rest is systemically available from the gastrointestinal tract) , the maximal systemic exposure would still be approximately 59 times lower than the one reported with oral niclosamide at the approved dose (Yomesan® chewable tablets) of 2 g/day, based on the reported 10% bioavailability of oral niclosamide, i.e. 200 mg day^{-1} / 3.4 mg day^{-1} = 58.8 [4]). Accounting for the 0.25 mg of niclosamide via nasal application, the systemic exposure would be 55 times lower (150 µL of 0.1% niclosamide per nostril).

At the highest concentration proposed in the dose escalation scheme (6 mL at a concentration of 1% twice daily), per day the maximum systemic exposure would still be approximately 2 times lower compared with the 2 g/day dose of niclosamide given orally (50.40 mg x 2 = 100.8 mg day^{-1}; 200 mg day^{-1}/100.8 mg day^{-1} = 1.98). Under such conditions, the Sponsor concludes that subjects are not put at unacceptable risk and that the anticipated AEs resulting from systemic exposure of the test product can be reasonably defined from the safety data collected with oral niclosamide. Accounting for the 5 mg of

niclosamide via nasal application, the systemic exposure would be 1.89 times lower (150 µL of 1% per nostril, BID).

With regard to local pulmonary side effects, the Sponsor refers to a recent publication [27] reporting the development of an inhalable formulation of niclosamide for the treatment of lung infections in patients with cystic fibrosis. After having confirmed the lack of cytotoxicity on bronchial epithelial cells *in vitro* (niclosamide concentrations tested up to 1,000 µM), the authors investigated the acute *in vivo* toxicity of niclosamide solutions (10, 30 or 100 µg niclosamide in 50 µL) intra-tracheally administered by a MicroSprayer in Wistar rats ([28], 4 rats per dosage group; body weight 200 – 220 g). 24 hours after *in vivo* administration, bronchoalveolar lavage fluid was obtained for protein evaluation as an index of lung damage. This test did not reveal any significant difference, compared to the control group, in the samples from rats treated with the formulation up to the equivalent niclosamide dose of 100 µg/rat. Only in the 100 µg group, an increase in the number of macrophages and neutrophils compared to control was observed, but without signs of lung injury as no iNOS expression and no significant difference in COX-2 expression was observed. Both are markers of lung injury induced by mechanical (e.g. ventilation) or chemical (e.g. acids) stress [29–31]. Therefore, the amount of niclosamide exposed to the human lung in the starting dose should be below the human equivalent of 100 µg in the rat.

The calculation of the human equivalent to 100 µg in the rat would account for the following factors: *weight of rat = 210 g, km factor rat = 6; km factor human = 37.* In addition, with the specific inhalation device ( ) approx. 48% of the product in the device will at most reach the lungs.

The sponsor proposes to take a conservative approach for determining a starting dose by applying an additional safety factor of 3.4-fold on the derived NOAEL of 100 µg (corresponding to 3.4 mg/m^2) in rats, from the Costabile study. Furthermore, the inhalation solution will be diluted with saline solution by a factor of 10 from 1% to 0.1% solution.

Based on these considerations (see calculation below) a volume of 4 mL of a niclosamide solution 0.08 % would be considered as an adequate starting dose in humans following single administration by the inhalation route. Since the test product is the of niclosamide, the concentration in the proposed starting dose should be 0.1%, corresponding to 4.0 mg of niclosamide in a 4 mL fill volume. Accordingly, the proposed initial dose for inhalation, 4 mL of a 0.1% niclosamide , is considered safe based on available nonclinical and clinical information

Calculation of human equivalent to 100 µg in the rat with a 3.4-fold safety factor

Factors: weight of rat = 210 g, k_m factor rat = 6, k_m factor human = 37; weight of adult = 70 kg

For 100 µg: (0.1 mg/0.21kg) = 0.48 mg/kg, corresponding to 2.9 mg/m^2 (assuming a k_m factor of 6). Applying a safety factor of 3.4 on this dose (mg/m^2), the human equivalent dose would be 0.85 mg/m^2. For a 70 kg adult this would correspond to a total dose of

1.61 mg (assuming a k_m factor of 37); since only 48% of the total dose will reach the lung the total amount of drug in the nebulizer is 100/48 x 1.61 = 3.4 mg. 3.4 mg dissolved in 4 mL corresponds to a concentration of 0.08% of niclosamide corresponding to a concentration of 0.1% for the of niclosamide.

The formulation used for nasal atomization is the same than the one used for nebulization, UNI911 INHALATION 0.1% and 1%. The quantity of product sprayed into each nostril is however significantly lower than the amount nebulized into the lung. A soft, conical plug on the tip forms a seal with the nostril, preventing expulsion of fluid and loss of product during the procedure. Using the syringe plunger, the spray then atomizes the drug product into a fine mist of particles in size. The total volume sprayed is enough to homogeneously cover the entire nasal cavity, with the excess product being swallowed. Since topical niclosamide was tested at up to 4% on abraded skin of healthy volunteers without local tolerance issues, no safety concern is expected from this mode of administration. The small quantity swallowed is known to have very limited bioavailability (around 10%) and will not significantly affect the systemic exposure related to the nebulization procedure.

6 STUDY OBJECTIVES AND OUTCOME MEASURES

6.1 Objectives

This study is designed to assess the safety of UNI911 INHALATION in HVs following a single ascending dose and multiple dosing as preparation for a subsequent Phase II study which will assess safety and efficacy in adults with COVID-19.

The primary objective of the current study is to assess safety of UNI911 INHALATION in healthy volunteers.

6.2 Outcome Measures

6.2.1 Safety Variables

Safety is assessed through the following parameters variables:

- AEs reporting (see section 11.2)
- General safety assessments
- General physical examination
- Vital signs
- Clinical laboratory analysis, including urinalysis, hematology, and serum chemistry (see also Appendix 16.3)
- ECGs
- Vital capacity
- Static volume (TLC)
- Diffusion capacity (DCO)
- Expiratory volume (FEV1)
- Fraction of expiratory nitric oxide (FeNO) tests
- Resting pulse oximetry
- CPET with pulse oximetry

6.2.2 Efficacy Variables

In this study, HVs are exposed to the IP; therefore, no assessment of efficacy is feasible.

6.2.3 PK Variables

Cohort 1-4: Blood samples for PK analysis will be collected: Pre-dose, ½, 1, 1½, 2, 3, 6, 12, and 24 hours after dosing

Cohort 5: Blood samples for PK analysis will be collected on Day 0 in the morning at pre-dose, 1, 3, and 6 hours after dosing and in the evening at pre-dose, 1 and 3 hours after dosing. No PK samples will be collected on Day 1. On Day 2, blood samples for PK analysis will be collected at ½, 1, 1½, 2, 3, 6, 12, and 24 hours after dosing.

6.2.4 Endpoints

Primary endpoint

- AE frequency in each cohort and treatment group
- Change from baseline for all safety variables measured and frequency of out of range values

PK Endpoints (Key PK Parameter)

- Maximum concentration of active drug molecules in blood (C_{max})
- Time to reach maximum level (T_{max})
- Area Under the Curve of drug level in blood versus time (AUC)
- Half-life ($T_{½}$)

Exploratory endpoint

- Oropharyngeal microbiome changes (will be analyzed *post hoc*)

7 TRIAL DESIGN

This is a single center, interventional, double-blinded (open label for the first sentinel subject within each cohort), placebo-controlled, Phase 1 study to assess the safety and explore PK parameters of UNI911 INHALATION in HVs. The study consists of five cohorts, which are started one after the other, each after consultation of the Safety Monitoring Committee (SMC; see section 13.3)). Each cohort starts only if the previously collected data do not give raise to safety concerns.

44eligible HVs are enrolled in 5 sequential cohorts for dose finding. 27 of these receive the IP, and 8 the placebo. The study is partly conducted in an open-label design (1st subject in cohort 1-4), and partly double blinded (subsequent subjects in cohorts 1-4 and all subjects in cohort 5).

- Cohort 1 : 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (4 mL, 0.1%, equaling 3.4 mg niclosamide) and nasal spray (2 x 150 µL, 0.1%, i.e. once per nostril, totaling 0.25 mg niclosamide) and 2 to receive placebo
- Cohort 2: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (1 mL, 1%, equaling 8.4 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo.
- Cohort 3: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (3 mL, 1%, equaling 25.2 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo.
- Cohort 4: 9 healthy volunteers, 7 to receive a single dose of UNI911 INHALATION (6 mL, 1%, equaling 50.4 mg niclosamide) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide) and 2 to receive placebo.
- Cohort 5: 8 healthy volunteers, 6 to receive five doses of UNI911 INHALATION (6 mL, 1%, equaling 50.4 mg niclosamide per dosing and 252 mg in total) and nasal spray (2 x 150 µL, 1%, i.e. once per nostril, totaling 2.5 mg niclosamide per dosing and 12.6 mg in total) dosed BID for 2.5 days and 2 to receive placebo.

Screening and enrollment for the cohorts will be initiated in parallel, while dosing will be done sequentially. Screening may be performed up to 21 days before initiation of study treatment. Dosing will begin with cohort 1. Once data from a minimum of 8 subjects (i.e., at least 6 subjects on active treatment) from cohort 1 is available, the safety parameters will be assessed by a Safety Monitoring Committee (SMC) before initiating dosing of subjects in cohort 2. Similarly, the SMC will review the data from all exposed subjects and a minimum of 8 subjects in cohorts 2, 3 and 4 before opening for dosing in the subsequent cohorts3, .

For cohorts 1-4, one subject will be dosed with UNI911 INHALATION (open label) the first day and followed for 24 hours while admitted at the clinic to assess safety of the new dose.

For cohort 5, patients will receive a total of 5 administrations and will stay at the trial site for 3 days, including overnight. In cohort 5, all patients will be blinded and randomized.

If safety concerns are observed (more than 2 subjects with Grade 3 AEs according to the scale CTCAE version 5 [33]) the SMC will adjudicate. If no safety concerns are observed or the SMC judges it safe to continue dosing, the remaining subjects in the cohort can be randomized and dosed (double-blinded) with an interval of at least one hour (dosing

interval only required for cohorts 1-4). After completion of the study, the SMC will assess safety and review PK data to recommend a dose and a treatment regimen proposed for a planned Phase 2 study in hospitalized COVID-19 patients.

For cohort 1, 2 , 3, and 4 the treatment will be a single ascending dose of UNI911 INHALATION or a placebo administered *via* inhalation and nasal spray by qualified study staff, after which the subject will be followed for 24 hours in the clinic and return for a final check 48 hours after dosing.

For cohort 5 the treatment will be multiple doses of UNI911 INHALATION or a placebo administered via inhalation and nasal spray by qualified study staff, after which the subject will be followed for 24 hours in the clinic and return for a final check 48 hours after last dose.

A general physical examination and serum chemistry and hematology sampling as well as urinalysis will be performed at screening, pre-dose and 24 and 48 hours after last dose. Subjects will be requested not to engage in strenuous physical exercise in the 72 hours preceding blood sampling to avoid abnormal values caused by exercise. If the first screening visit is conducted more than 3 days before first dosing, the subject must come to the clinic 1 - 3 days before dosing for an oropharyngeal swab (to confirm no infection with SARS-CoV-2[1]) and sampling for serum chemistry, hematology and urinalysis.

In terms of respiratory function, safety will be assessed on the basis of spirometry (vital capacity and FEV1) as well as pulse oximetry performed at

- Vital capacity and FEV1:
  - Cohort 1-4: pre-dose, 1, 3, 6, 12, and 24 hours after dosing.
  - Cohort 5:
    - at Day 0 and Day 1: pre-dose, 1, 3 hours after morning and evening dosing
    - At Day 2: pre-dose, 1, 3, 6, 12, and 24 hours after morning dosing

- DCO:
  - Cohort 1-4 : pre-dose, 1 and 3 hours after dosing
  - Cohort 5: not applicable (will be done at screening and on Day 4, see below)

1 In a recent guideline [34], specific precautions are recommended for the use of nebulizers in COVID-19 patients in order to avoid spreading the virus. In this study, only subjects with a negative SARS-CoV2 test will be eligible, thus, nebulizers can be used according to their respective manuals. In subsequent studies in COVID-19 patients, spacers and other safety measures will be applied in accordance with current recommendations.

In addition, FEV1 (including reversibility), TLC, DCO, and FeNO will also be measured and a CPET with pulse oximetry will be conducted during the screening period (between ICF signing and dosing) and on Day 2 after dosing[1] for cohort 1-4 and on Day 4 for cohort 5.

ECGs will be captured at

- Cohort 1-4:
  - Screening
  - Day 0: pre-dose, 3, 6, and 24 hours after dosing
- Cohort 5:
  - Screening
  - Day 0: Pre-dose, 3 hours after the morning dose
  - Day 2: Pre-dose, 3, 6, and 24 hours after dosing

Vital signs (systemic blood pressure, pulse, respiratory rate (RR), and body temperature) will be measured at:

- Cohort 1-4: screening, pre-dose, 1, 3, 6, 12, 24, and 48 hours post dose.
- Cohort 5:
  - Screening
  - Day 0 and Day 1: pre-dose, 1, 3 hours after morning and evening dosing
  - Day 2: pre-dose ,1, 3, 6, 12, 24 and 48 hours after morning dosing

AEs will be collected from the time of first dose and through-out the study period. Finally, an oropharyngeal swab for detection of viruses and bacteria will be taken pre-dose and 48 hours after (last) dose for *post hoc* exploratory analysis of potential changes in the microbiome.

Eligible HVs must not be smokers, should be in good general health and have a normal medical history excluding any chronic disease as per the investigator's judgment as well as minimum 80% of predicted lung function, including FEV1, TLC, DCO, and CPET with

1 The day of dosing is designated as Day 0

pulse oximetry. Vital signs, ECG and X-ray must not be clinically significantly abnormal as well (see eligibility criteria for full details in sections 8.2 and 8.3).

8 POPULATION

8.1 Number of Subjects

44healthy volunteers will be enrolled such a way that 7 HVs are treated with each of the four doses of UNI911 INHALATION once-daily for cohorts 1-4, 6 HVs are treated with the recommended dose for the multiple dose cohort and 8 with placebo. Subjects are randomized to a treatment and a placebo group (Table 1). Only subjects who fulfill all criteria for inclusion and none of the criteria for exclusion, will be included into this study. This sample size is justified in section 12.1.

Table 1: Subjects by cohort and treatment group

Cohort	Treatment	Placebo	Total
Cohort 1	7	2	9
Cohort 2	7	2	9
Cohort 3	7	2	9
Cohort 34	7	2	9
Cohort 5	6	2	9
Total	**34**	**10**	**44**

8.2 Inclusion Criteria

Subjects who meet all of the following criteria are eligible to participate in this study:

(1) Signed Informed Consent Form (ICF)

(2) Male or nonpregnant and nonlactating female who is abstinent or agrees to use effective contraceptive methods throughout the course of the study. Females must have a negative urine beta-human chorionic gonadotropin hormone (hCG) pregnancy test prior to dosing (Women who are postmenopausal[1] or who had tubal ligation/hysterectomy do not need to have a pregnancy test done and do not need to

1 Menopause is defined as the time when there have been no menstrual periods for 12 consecutive months and no other biological or physiological cause can be identified.

agree to use contraception).
Acceptable birth control methods are the following:

- Intrauterine device in place for at least 3 months
- Stable hormonal contraceptive for at least 3 months prior to dosing and continuing through study completion
- Surgically sterilized partner

(3) ECG without clinically significant abnormalities (including QTcF < 450 ms)

(4) Age ≥ 18 and < 65 years at the time of signing ICF

(5) Normally active and in good health by medical history and physical examination

(6) Minimum 80% of predicted lung function, including FEV1 after beta2-agonist, TLC, DCO, and CPET with pulse oximetry

(7) Chest X-ray without clinically significant abnormalities

8.3 Exclusion Criteria

Subjects who meet any of the following criteria are not eligible to participate in this study:

(1) Enrollment in an UNI911 study in the previous 6 months

(2) Clinically significant allergy (as judged by the investigator) or history of significant adverse reaction to niclosamide or related compounds, to any of the excipients used,

(3) Underlying condition that may interfere with inhalation of the IP

(4) Current acute or chronic condition (including COPD, asthma, or other severe respiratory disease, CV disease, diabetes mellitus, obesity, malignant and autoimmune diseases) unless considered clinically irrelevant and stable by the investigator

(5) Renal impairment (eGFR < 60 mL/min/1.73m²) or hepatic impairment (as judged by the investigator)

(6) The presence of a condition which renders the subject “vulnerable“ as defined by GCP or of a condition the investigator believes would interfere with the ability to provide informed consent, or comply with study procedures/instructions, or that might confound the interpretation of the study results or put the subject at undue risk

(7) Smoke or regular use of any form of nicotine product including e-cigarette, snuff, chewing tobacco, nicotine gum, etc., during the study period and for the previous 6 months

(8) Known difficulty undergoing venipuncture or poor venous access

(9) Whole blood donation or loss (> 400 mL) within 90 days before the dosing of IP

(10) History of any malignancy except subjects with adequately treated basal cell / squamous carcinomas of the skin

(11) Consumed alcohol in the 24 hours prior to dosing

Prior or concomitant therapy:

(12) Any systemic and inhaled therapies 5 half-lives prior to dosing (hormone replacement therapy for postmenopausal women and hormonal anticonception are allowed).

(13) Participation in any clinical trial 90 days prior to dosing of UNI911 INHALATION or placebo

Should a potential subject fail screening, the person can be re-screened and included, if he/she conforms to all inclusion and none of the exclusion criteria at a later stage.

8.4 Subject Withdrawal from the Study

Subjects may opt to withdraw from the study at any time, without being obliged to disclose their reason. The investigator will explain the importance of remaining in the study and make reasonable effort to clarify the reason for withdrawal.

8.5 Subject Withdrawal from Study Treatment

Subjects may be discontinued from treatment or from the study at the request of the investigator or by UNION therapeutics, especially for safety reasons. If a subject does not complete IP administration, reasonable efforts will be made to complete all protocol-specified assessments. Withdrawal from study treatment is mandatory in the case of any of the following:

- Safety of subject is placed at risk as assessed by the investigator
- Symptoms of unacceptable toxicity of the IP as assessed by the investigator. This includes, but is not restricted to, the occurrence of an SAE or severe AE where a causal relationship to study procedures cannot be ruled out.
- The subject does not comply with study procedures

8.6 Study Discontinuation

UNION therapeutics may terminate the study at any time. Due to the monocentric nature of the study, termination of the study site also results in termination of the whole study. Reasons for terminating the study or the site include, but are not limited to, the following:

- The incidence, severity or outcome of AEs in this or other studies using the same IP indicate a potential health hazard to subjects (if 3 or more subjects experience an AE

with a grade of 3 or higher according to CTCAE [33], the study will be put on hold and the data presented to the SMC for a decision on whether and how to proceed)

- The SMC (see section 13.3) recommends discontinuation of the study after data review
- Subject enrollment is unsatisfactory, and no meaningful results can be expected in spite of all reasonable recruitment efforts.
- Data recording is inaccurate or incomplete in spite of reasonable efforts undertaken in the frame of monitoring
- The investigator does not adhere to the protocol or applicable legislation or regulatory guidelines in conducting the study
- The Independent Ethics Committee (IEC) or the competent authority terminate or suspend approval for the study or the investigator

Any interruption or discontinuation will be immediately reported to the competent authority and to the IEC.

8.7 Concomitant Therapy

Eligible HVs should not be expected to use receive any other pharmacological therapy than the IP. However, subjects may use hormonal contraception or take specific medications as prophylaxis, or medications may be required for the treatment of AEs. The use of any medication by a subject during the course of the study and the reason for its use is documented in the source documents and the case report form (CRF), including 30 days prior to screening. The use of prohibited medications (Table 2) is recorded as a protocol deviation (PD, section 13.8).

Table 2: Prohibited or restricted concomitant therapies

Therapy	Restriction
Any investigational product	90 days prior to inclusion from participation in any other clinical trial
Approved medicinal products	Any systemic and inhaled therapies 5 half-lives prior to day 0. Hormone replacement therapy for postmenopausal women and hormonal anticonception are allowed.

Non-drug therapies are documented in the same way as pharmacotherapy. If such a therapy is expected to interfere with further participation in the trial, the investigator will consider discontinuation of the subject.

9 STUDY TREATMENT

9.1 Description of Study Drug

UNI911 INHALATION consists of nebulizer solution of niclosamide .
(The technical name UNI911 Nebulizer Solution is used in this section to describe the IP otherwise referred to as UNI911 INHALATION in the rest of the protocol.)

Subjects in the active treatment group Cohort 1 are exposed to UNI911 Nebulizer Solution 0.1%, which will be administered by pulmonary inhalation and as a nasal spray.

Subjects in the active treatment groups Cohorts 2-5 are exposed to UNI911 Nebulizer Solution 1%, which will be administered by pulmonary inhalation and as a nasal spray.

Nota bene: Description of the delivery devices is appended to this protocol in Annex 16.2 B and more details will be provided in the separate Pharmacy manual.

Table 3 shows the characteristics of the IP.

Table 3: Characteristics of the IP

Product name:	UNI911 Nebulizer Solution 1%
Dosage form:	Aqueous solution
Unit dose strengths:	Cohort 1: 4 mL of a 0.1 % solution + 150 µL of a 0.1 % solution in each nostril Cohort 2: 1 mL of a 1.0% solution +150 µL of a 1.0% solution in each nostril Cohort 3: 3 mL of a 1.0% solution + 150 µL of a 1.0% solution in each nostril Cohort 4 and 5: 6 mL of a 1.0% solution + 150 µL of a 1.0% solution in each nostril Placebo: same composition with adjustment of pH and osmolarity without drug substance
Route of Administration:	Inhalation by nebulization + nasal spray applicator
Treatment instructions:	Cohorts 1, 2, 3, 4: Single administration Cohort 5: BID for 2.5 days
Physical description:	IP: Clear red-orange liquid Placebo: Clear slightly yellow liquid*
Source of procurement:	UNION therapeutics A/S

* It is acknowledged that this difference might enable identification of the IP. The selection of placebo is justified in section 9.4.

9.2 Packaging, Labelling, and Storage of IP

The IP will be supplied from the IP producer to the trial site in 10 mL clear glass vials containing 7 mL of UNI911 Nebulizer Solution 1% and 10 mL clear glass vials containing 7mL of UNI911 Placebo Nebulizer Solution.The vials must be stored in a refrigerator at (2-8 °C) in a place with restricted access and without access for blinded study staff. Storage conditions, packaging and labelling of the IP are specified in a separate Pharmacy Manual.

9.3 Dose and Administration

The UNI911 Nebulizer Solution 1% will be diluted extemporary to obtain a 0.1% solution (the dilution procedure will be specified in the Pharmacy manual)

Subjects are exposed to inhalation of ascending doses of the IP.

Cohort 1: 4mL of 0.1 % solution (corresponding to 3.4 mg niclosamide) and nasal administration of 2 times 150 µL, 0.1%, one administration to each nostril (totaling 0.25 mg niclosamide), will be applied

Cohorts 2, 3, 4: 1, 3 and 6 ml of 1% solution (corresponding to 8.4 mg, 25.2 mg, and 50.4 mg of niclosamide) and nasal administration of 2 times 150 µL 1%, one administration to each nostril (totaling 25 mg niclosamide), will be applied.

Maximum dose to be tested in cohort 5 is 6 mL UNI911 INHALATION 1% twice daily for 2.5 days (5 doses), BID on two first days and morning dose on day 2. Table 4 shows the single and total doses for the five cohorts.

Table 4: Medications and schedule for administration for each cohort.
This table shows only active treatment groups.

Cohort	**1**	**2**	**3**	**4**	**5**
Treatment Schedule	Single	Single	Single	Single	Multiple
Number of Administrations	1	1	1	1	5
IP Single and Total Dose* (Inhalation)	4 mL0.1% / 3.4 mg	1 mL1% /8.4 mg	3 mL1%/25.2 mg	6 mL 1% /50.5 mg	6 mL1% /50.5mg 30 mL/252mg

Cohort	1	2	3	4	5
IP Single and Total Dose* (Nasal**)	2 x 150µL 0.1% 0.25 mg	2 x 150µL 1% 2.5 mg	2 x 150µL 1% 2.5 mg	2 x 150µL1% 2.5 mg	2 x 150µL1%2.5 mg 10 x 150µL/25 mg
Duration of Treatment	1 d	1 d	1 d	1 d	2.5 d

* Active treatment groups only

** Administered once per nostril

Throughout the study, both IP are administered by qualified study staff. Each treatment is assigned to a specific subject by randomization number.

Inhalation is performed by using an EN 13544-1 certified nebulizer by . Permissible nebulizer models have been tested nebulizer systems with a reference solution according to the European Standard prEN13544-1, resulting in standardized information, including (1) description of the nebulizer system which includes the flow rates and volume fills at which tests were made; (2) rate of aerosol output and total aerosol output; (3) droplet size distribution curve from which the median size and spread, and per cent aerosol mass within any given range can be obtained ( ).

The nebulizers are used according to the manual instruction provided by Site staff is trained to use the system. The intranasal mucosa is treated by nasal spray by using . Site staff is trained to use the system.

9.4 Blinding

Of 44 subjects, 40 are treated in a double-blinded manner, whereas 4 are treated in an open label manner.

In order to speed up the initiation of this trial due to the ongoing pandemic, no individual subject kits are prepared. Instead IP and placebo are prepared for each subject at the site as described in section 9.3, the Pharmacy Manual. For the same reason, the placebo group receives a solution which also contai ut differs from the IP with regard to color and taste. Addition of dies and taste masking agents to the placebo could lead to AEs since there are no dies and taste additive that are approved for inhalation. Thus, there is specific risk that both subjects and investigators will figure out the respective assignment to treatment groups. The following precautions are taken in order to mitigate this risk (based on suggestions by [35]):

- The IMP will be stored without access for blinded study staff. The nebulizer and the Nasal device will be filled with the appropriate solution and volume

according to a randomization list by unblinded staff at the clinical site. Blinded study staff should not have direct contact with the IP (Details of the blinding will be described in the Pharmacy Manual).

- Subjects will be instructed by the study staff not to discuss any characteristics (e.g., the appearance and taste) of the study drug with blinded study staff or other research subjects.
- During waiting, treatment, and assessment periods, reasonable efforts are made to isolate subjects from each other. Conversations between subjects, which can be a source of undesired unblinding [35], are thereby prevented.

The randomization list will be secured in a locked cabinet and/or an electronic file with restricted access to only designated unblinded study staff.

The Principal Investigator will break the blind in case of emergency. Sealed code break envelopes will be held by the clinical site. In such an emergency, the study staff will only be breaking the blind for the subject involved. .

If the code is broken the envelope must be signed and dated by the individual who broke the code, and information entered into the subject's source documents, explaining the reason and date that it was opened, identity of the person who authorized the code break, and documenting the identity of the study product allocated to the subject. This must be countersigned by the Investigator.

9.5 Method of Assigning Subject to Study Treatment

Five cohorts with two treatment groups are defined. Enrolment will be done sequentially for one cohort after the other. Within each of the cohorts, subjects are randomized to treatment groups described in section 12.2.

9.6 Measures of Treatment Compliance

The IP is administered by unblinded qualified study staff at the trial site. In cohorts 1- 4, subjects stay at the site overnight, and come back only once on day 2. In cohort 5, subjects stay at the site 4 days, including overnight, and come back once on day 4.

As subjects are only dosed while admitted at the site, no specific measurement of treatment compliance is required. The subjects will not discuss the study drug with blinded study staff or other research subjects.

9.7 Investigational Product Accountability and Retention

All IP required for completion of this study is provided by UNION therapeutics or an authorized designee. The site is to acknowledge receipt of IP indicating content and

condition of the shipment. Damaged supplies are to be reported and to be replaced. The study center will maintain accurate records of dispensed, returned or destroyed IP. The study monitor is responsible for verifying drug accountability records at the study center. Inventory records are made available for audits and inspections by UNION therapeutics or its authorized representatives or by regulatory authorities, respectively. The investigator ensures that the IP is used in accordance with this protocol.

10 DESCRIPTION OF STUDY PROCEDURES

10.1 Procedures

Study participation lasts for approximately 3 days in cohort 1 – 4 and 5 days in cohort 5, excluding screening (Table 5 and Table 6).

Table 5: Schedule of study procedures in cohort 1 - 4

Study Procedures	Screening (Day -21 to -1)	Pre-dose D0 (-2-0 hours)	Dosing (Day 0)	+1 hour D0	+3 hours D0	+6 hours D0	+12 hours D0	+24 hours D1	Day 2
Informed consent (before any study procedures)	X								
Verify subject meets inclusion and exclusion criteria	X	X							
Admitted at clinic		X	X	X	X	X	X	X	
Record relevant medical history and demographic data (incl. height and weight)	X	X[1]							
Collect sample for urinalysis (by dipstick)	X							X	X
Collect urine sample for pregnancy test (WOCBP only)	X	X							
Collect blood samples for hematology and chemistry	X							X	X
Collect oropharyngeal swab to test for SARS-CoV-2[2]	X								
Collect oropharyngeal swab for post hoc microbiome analysis		X							X
Record medications (incl. 30 days prior to screening)	X	X	X	X	X	X	X	X	X
Record vital signs	X	X		X	X	X	X	X	X
Perform physical examination[5]	X	X						X	X

Study Procedures	Screening (Day -21 to -1)	Pre-dose D0 (-2-0 hours)	Dosing (Day 0)	+1 hour D0	+3 hours D0	+6 hours D0	+12 hours D0	+24 hours D1	Day 2
Capture ECGs (12-lead, in triplicate, 1 minute apart)	X	X			X	X		X	
Collect blood samples for PK analysis[3]		X		X	X	X	X	X	
Assess lung function by spirometry (vital capacity, FEV1)		X		X	X	X	X	X	
Assess lung function by DCO		X		X	X				
Assess lung function (II) (FEV1 with reversibility, TLC, DCO, FeNO)[4]	X								X
Perform resting pulse oximetry		X		X	X	X	X	X	
Perform CardioPulmonary Exercise Testing (CPET) with pulse oximetry[4]	X								X
Administer IP			X						
Collect adverse events			X	X	X	X	X	X	X

1 In case of new history since screening

2 If first screening visit is conducted more than 3 days before dosing, the subject must come to the clinic 1-3 days before dosing for an oropharyngeal swab (to confirm no infection with SARS-CoV2) and sampling for serum chemistry and hematology and urinalysis

3 PK samples will be collected at pre-dose, ½, 1, 1½, 2, 3, 6, 12, and 24 hours after dosing in cohorts 1 – 3

4 Baseline lung function and a CPET will be conducted during the screening period (between ICF signing and dosing) and on Day 2 after dosing. Before attending the CPET test, do not eat a heavy meal for 2 hours before, do not take alcohol for 4 hours before, do not take vigorous exercise for 30 min before, do not wear any tight clothing that may restrict your breathing.

5 At screening, a full physical examination will be conducted. At all subsequent timepoints, an abbreviated physical examination is permissible

Table 6: Schedule of study procedures in cohort 5

Study Procedures	Screening (Day -21 to -1)	Pre-dose D0 (-2-0 hours)	Dosing (Day 0)[2]	+1 hour D0	+3 hours D0	Predose Day 1	Dosing Day 1 [2]	+1 hours D1	+3 hours D1	Pre dose Day 2	Dosing Day 2 [3]	+ 1 hour D2	+3 hours D2	+6 hours D2	+12 hours D2	+24 hours D3	+48h D4
Informed consent (before any study procedures)	X																
Verify subject meets inclusion and exclusion criteria	X	X[9]															
Admitted at clinic		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Record relevant medical history and demographic data (incl. height and weight)	X	X[1,9]															
Collect sample for urinalysis (by dipstick)	X															X	X
Collect urine sample for pregnancy test (WOCBP only)	X	X[9]															
Collect blood samples for hematology and chemistry	X															X	X
Collect oropharyngeal swab to test for SARS-CoV-2[7]	X																
Collect oropharyngeal swab for post hoc microbiome analysis		X[9]															X
Record medications (incl. 30 days prior to screening)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record vital signs	X	X		X	X	X		X	X	X		X	X	X	X	X	X
Perform physical examination[8]	X	X[9]														X	X

Study Procedures	Screening (Day -21 to -1)	Pre-dose D0 (-2-0 hours)	Dosing (Day 0)[2]	+1 hour D0	+3 hours D0	Predose Day 1	Dosing Day 1 [2]	+1 hours D1	+3 hours D1	Pre dose Day 2	Dosing Day 2 [3]	+ 1 hour D2	+3 hours D2	+6 hours D2	+12 hours D2	+24 hours D3	+48h D4
Capture ECGs (12-lead, in triplicate, 1 minute apart) [4]	X	X			X					X			X	X		X	
Collect blood samples for PK analysis[5]		X		X	X					X	X	X	X	X	X	X	X
Assess lung function by spirometry (vital capacity, FEV1)		X		X	X	X		X	X	X		X	X	X	X	X	
Assess lung function (II) (FEV1 with reversibility, TLC, DCO, FeNO) [6]	X																X
Perform resting pulse oximetry		X		X	X	X		X	X	X		X	X	X	X	X	
Perform CardioPulmonary Exercise Testing (CPET) with pulse oximetry [6]	X																X
Administer IP			X				X				X						
Collect adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 In case of new history since screening

2 BID dosing; time between dosing 8 hours at minimum

3 Dosing in the morning only

4 ECG at Day 0 will be captured after the morning dose only

5 At Day 0 PK samples will be collected in the morning at Pre dose, 1, 3, 6 hours after dosing and in the evening at pre-dose, 1, 3 hours after dosing.
No PK samples will be collected on Day 1. At day 2 PK samples will be collected at pre-dose, ½, 1, 1½, 2, 3, 6, 12, 24 and 48 hours after dosing

6 FEV1 (including reversibility), TLC, DCO, and FeNO will be measured and a CPET with pulse oximetry at the screening period (between ICF signing and dosing) and on Day 4 after dosing. Before attending the CPET test, do not eat a heavy meal for 2 hours before, do not take alcohol for 4 hours before, do not take vigorous exercise for 30 min before, do not wear any tight clothing that may restrict your breathing.

Study Procedures	Screening (Day -21 to -1)	Pre-dose D0 (-2-0 hours)	Dosing (Day 0)[2]	+1 hour D0	+3 hours D0	Predose Day 1	Dosing Day 1 [2]	+1 hours D1	+3 hours D1	Pre dose Day 2	Dosing Day 2 [3]	+ 1 hour D2	+3 hours D2	+6 hours D2	+12 hours D2	+24 hours D3	+48h D4

7 If first screening visit is conducted more than 3 days before dosing, the subject must come to the clinic 1-3 days before dosing for an oropharyngeal swab (to confirm no infection with SARS-CoV2) and sampling for serum chemistry and hematology and urinalysis

8 At screening, a full physical examination will be conducted. At all subsequent timepoints, an abbreviated physical examination is permissible

9 Verification of eligibility criteria, record of medical history and demographic data, collection of urine samples for pregnancy tests, physical examination, and collection of an oropharyngeal swab for *post hoc* microbiome analysis are only conducted at morning pre-dose.

10.2 Sample Handling

Venous blood samples are taken in the frame of the safety and PK assessments (sections 12.6 and 6.2.3). Blood sampling is carried out according to the clinical routine of the trial site. The minimum amounts required by the laboratory are sampled, observing the maximum permissible amounts defined by applicable guidelines [36, 37]. Total blood volume collected per subject will be approximately 110 mL in cohorts 1-4 and 150 mL in cohort 5. Furthermore, swab samples are collected. Collection and analysis of these samples is described in Appendix 16.2.

Blood samples are analyzed by the local laboratory, while SARS-CoV2 swabs and microbiome samples are analyzed at the microbial lab (appendix 16.1). Blood samples and SARS-CoV-2-swabs will be analyzed continuously to provide results during the study, while microbiome samples will be stored frozen and analyzed in one batch, when all samples have been collected. All remaining samples are destroyed at the end of the study.

Urine samples will be collected for urinalysis and pregnancy tests (for WoCBP). These will be analyzed at the site and discarded after analysis.

This section does not apply to additional samples that might be taken in the frame of clinical routine, e.g. for the treatment of AEs or routine diagnoses.

11 SAFETY

Safety will be evaluated by presenting summaries of treatment emergent AEs (TEAEs), clinical laboratory evaluations (chemistry, hematology, microbiology), vital signs, oximetry, airflow measurements, physical examinations, and imaging techniques. Furthermore, subjects are encouraged to self-report AEs.

11.1 Definition of Safety Relevant Events

11.1.1 Adverse Events

GCP defines an adverse event (AE) as "*any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (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*" [11]. Medical conditions present at baseline are considered medical history, and

not AEs. However, any worsening of a pre-existing medical condition during the AE reporting period (see section 11.1.10) is to be reported as an AE.

11.1.2 Treatment Emergent Adverse Events

"*Treatment emergent adverse events (TEAEs) are undesirable events not present prior to medical treatment, or an already present event that worsens either in intensity or frequency following the treatment*" [38]. AE reporting starts with the start of treatment; thus, all AEs reported in this study are also TEAEs, except for those members of the ITT population where no treatment was administered for any reason.

11.1.3 Adverse Drug Reaction

GCP defines adverse drug reactions (ADR) as "*all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out*" [11].

11.1.4 Serious Adverse Event

GCP defines an AE as "serious" if it "*results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect, or is an important medical event as judged by the investigator*" [11]. "Life-threatening" means that the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe [39]. Important medical events should be considered as SAEs even if they do not meet any of these conditions if they jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above [39].

For the reporting of SAEs, the rules defined in section 11.2 apply.

11.1.5 Unexpected Adverse Event

An AE is defined as "unexpected", if "*the nature or severity ... is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product)*" [39].

Unexpected AEs are defined as Suspected Unexpected Serious Adverse Reactions (SUSARs) if they fulfill any criterion of an SAE (section 11.1.4) and are also ADRs (section 11.1.3). SUSARs will be communicated to all concerned regulatory authorities (section 11.2). If the investigator has assessed that there is no reasonable possibility that the SAE has been caused by the IP, but the sponsor decides that there is a reasonable possibility of a causal relationship, the event is reported as a SUSAR.

11.1.6 Adverse Event of Special Interest

An AESI is a noteworthy event for the particular study drug that may deserve close monitoring. AESIs can be serious or non-serious and can include events that might be potential precursors or prodromal symptoms for more serious medical conditions in susceptible individuals.

The AEs of Special Interest for this protocol are pre-defined as follows:

- Prolonged (more than 2 hours after end of nebulization) or severe (needing medical management) coughing due to the inhalation procedure
- Bronchospasm due to the inhalation procedure
- Any sign or symptom in the nasopharyngeal area, the severity of which would require medical management

For AESIs, the Investigator is required to complete the Adverse Event Form on the CRF and follow the SAE reporting procedures in Section 11.1.2 even if the event is considered non-serious according to the usual regulatory criteria as listed in section 11.1.4. Prompt and appropriate medical action is taken, if necessary. The safety of subjects is the first priority. AESIs will be monitored until the Investigator and Sponsor agree that the event is satisfactorily resolved.

Pharmalex is to be informed about the final outcome of the event, e.g. by sending a revised or updated AE Form, if appropriate.

Furthermore, demographics, medical history, previous and concomitant therapies, and AE pages of the CRF must be completed and available for review at the time of the report.

11.1.7 Intensity of Adverse Events

AEs are graded as mild, moderate, or severe, as defined in Table 7.

Table 7: Grading of AEs

Severity	Definition
Mild	Minor and does not cause significant discomfort to subject or change in activities of daily living (ADL); subject aware of symptoms but symptoms easily tolerated
Moderate	Causes inconvenience or concern to subject and interferes with ADL, but subject able to continue with ADL
Severe	Significantly interferes with ADL and subject is incapacitated and/or unable to continue with ADL

11.1.8 Relationship of Adverse Events to Study Drug

The relationship of each AE to the IP must be determined by a medically qualified individual according to the following definition Table 8. AEs with a "possible", "probable" or "definite" relationship are summarized as "related". In case of a possible, probable, or definite relationship to test drug, the relationship to test drug should be indicated as "Yes" in the AE form of the CRF.

Table 8: Classification of the causal relationship between an AE and the IP

Category		Definition
Unrelated		This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under possible, probable, or definite.
Related	**Possible**	The AE follows a reasonable temporal sequence from the time of study product administration but could have been caused by the study participant's clinical state or other modes of therapy administered to the participant.
	Probable	The AE follows a reasonable temporal sequence from the time of study product administration, abates upon discontinuation of the study product and cannot be reasonably explained by the known characteristics of the study participant's clinical state.
	Definite	The AE follows a reasonable temporal sequence from the time of study product administration, abates upon discontinuation of the study product and reappears when study product is reintroduced.

11.1.9 Treatment and Outcome of Adverse Events

In order to ensure safety and well-being of the subject, the investigator will take appropriate action. Possible treatment of AEs include (1) none; (2) discontinuation of the IP; (3) prescription of concomitant medication, described in more detail in section 8.7; (4) prescription of non-drug treatment like mechanical ventilation or O_2 therapy.

For each AE, the outcome is classified as: (1) recovered; (2) recovered with sequelae; (3) ongoing; (4) fatal; (5) unknown. "Unknown" is only permissible if reasonable efforts have been made to elucidate the fate of the subject. Stop dates are only recorded if the outcome is 1, 2 or 4.

11.1.10 Reporting Period for Adverse Events

The investigator ensures reporting of all AEs occurring during the reporting period. For each AE, the following information is recorded at minimum: start and stop dates (if not ongoing), intensity (severity), seriousness, causal relationship to the IP, action taken, and outcome. In order to avoid underreporting, the study personnel ask neutral questions in the frame of AE assessment (e.g. "*How are you feeling?*" or "*Have you noticed any changes in your health?*"). Furthermore, physical examinations, ECG, oximetry, recording of vital signs, X-ray or CT Scans and safety laboratory analyses are conducted. Changes in the current treatment are also assessed carefully in order to identify AEs in subjects. Clinically significant findings are reported as AEs or medical history, whatever applies.

AEs are documented from baseline (i.e., starting from the receipt of the first dose of the IP/placebo) until the last visit attended by the subject, both in the source data and in the CRF. Events prior to baseline are documented as medical history, including events emerging between screening and baseline. Worsening of medical history events will be reported as AEs. Ongoing AEs are evaluated and documented at the last visit. Follow-up of ongoing AEs is described in section 11.3. Premature discontinuation of treatment does not affect the reporting period.

11.2 Reporting of Serious Adverse Events

Any SAEs occurring during the reporting period (section 11.1.10) are reported by the investigator to UNION therapeutics and to the PV provider PharmaLex (section 16.1) within 24 hours after becoming aware of the SAE. UNION therapeutics or its authorized representatives support the investigator in compiling all necessary information and ensures that UNION therapeutics receives a report within 24 hours after notification.

UNION therapeutics will process the cases and evaluate if expedited reporting is needed. In this case, UNION therapeutics will submit the data to the regulatory authorities and ethics committees and to other investigators, if any, within 7 d for initial reports on life-threatening or fatal related events, and within 15 d for other related events.

SAE reporting involves completing and signing an SAE form in English by the investigator or his authorized representative. The original signed SAE report is archived in the investigator site master file. This form should provide comprehensive information about the event, including, but not limited to:

- Identification of the trial by name, protocol code and version and EUDRACT number
- A short description of the event and the reason why the event was categorized as serious. If possible, a diagnosis should be provided instead of symptoms or procedures.
- Subject identification number
- Basic demographic data
- Investigator's name and contact data, including the trial site

- Name of the IP
- Diagnostic procedures, laboratory tests and treatments
- Causality assessment
- Intensity (severity)
- Indication of either initial or follow-up report
- Outcome
- Statement of the investigator

Of these details, the following are to be provided in the report, as a minimum: subject identification, a short description, investigator's name, the IP, and a causality assessment have to be provided. SAEs are followed as described in sections 11.1.10 and 11.3. The timelines and procedures for follow-up reports are the same as those for the initial report. SUSARs require expedited reporting to regulatory authorities, ethics committees and investigators of the trial. Fatal or life threatening SUSARs have to be reported immediately, but in all cases within 7 calendar days after initial notification. A finalized report must be provided within 8 additional days. All other SUSARs must be submitted within 15 calendar days from that point in time when the sponsor receives initial notification. Pregnancies, if any (section 11.4), are not regarded as AEs but still reported by using the SAE form.

SAEs are to be reported by phone and e-mail (supplemented by fax in case of e-mail malfunction) to the following functions (contact data are provided in appendix 16.1):

- Pharmacovigilance
- Chief medical officer of the sponsor

For SAEs AESIs, and pregnancies the PV provider is to be informed immediately by fax or email. The event must be reported by facsimile or scan and sent by e-mail to the PV provider within 24 hours of knowledge of the event.

11.3 Follow-Up of Adverse Events

The investigator follows AEs occurring during the AE reporting period (section 11.1.10) with a clinically appropriate effort until the event resolves or is considered stable or the subject dies or withdraws consent for further evaluation, whatever occurs first. For subjects lost to follow-up during the reporting period with ongoing AEs, the investigator will make reasonable efforts to keep contacting the subject.

Any SAE judged by the Investigator to be related to the study treatment should be reported to the Sponsor regardless of the length of time that has passed since study completion.

11.4 Pregnancy

Female subjects of childbearing potential are eligible only if a negative pregnancy test has been carried out and they agree to use a medically acceptable method of contraception, or

to exercise abstinence, or if either tubal ligation or hysterectomy have been documented. It is extremely unlikely that a pregnancy will emerge during the 3-5 days of study participation. However, subjects are instructed to inform the investigator about any suspected or confirmed pregnancy. No specific measures are prescribed concerning the continuation of the study treatment, since only a single administration will be done. Study-related safety assessments are continued in case of a pregnancy.

All pregnancies are reported within 24 h as outlined for SAEs in section 11.2. Pregnancies are followed, using a predefined pregnancy form, until final outcome, including any premature termination. Live births are followed for a minimum of 30 d or until the first well-baby visit, whatever occurs first.

11.5 Other Safety-Relevant Data

Laboratory data (clinical chemistry and hematology) are assessed for additional safety monitoring (Table 11 in Appendix 16.3). If applicable, values are classified as "normal" *vs*. "abnormal" according to the standards of the local laboratory (16.1) at the time of the analysis, or according to the standard of the site. In the same way, the findings of the physical examination, vital signs, ECG data, oximetry, airflow measurements and images are assessed as "normal" or "abnormal" according to clinical routine.

Abnormal values are further classified as "not clinically significant (NCS)" or as "clinically significant (CS)" by the investigator under consideration of all available information. Clinically significant abnormal values are reported as medical history, if they are already present pre-dose, or as an AE if detected after dosing (including post dosing worsening of medical history events). No such classification is made for values without a predefined normal range; however, the investigator will generate medical history / AEs if a value is considered as significant. No separate medical history or AE is generated if the clinically significant abnormal value is considered to be part of another medical history / AE by the investigator.

12 STATISTICAL METHODS

12.1 Sample Size

The study is not powered for inferential statistics. This sample size is considered sufficient to meet the study objectives and to assess treatment safety but is not based on statistical power considerations. Table 9 shows the power of the study to detect AEs at least once.

Table 9: Incidence rates of AEs which can be expected to be observed at least once with a probability of 80%

Study Phase	Number of participants	Incidence of AEs
Treatment	34 HVs	7.3%
Placebo	10 HVs	22.75 %
Total	44 HVs	5.6%

12.2 Randomization

Screening and enrollment will be done sequentially for one cohort after the other. A randomization number will be assigned in ascending order to each eligible subject at Day 0 according to the randomization list by cohort. The first number of the cohort 1, 2 , 3 and 4 will always be active (open label) and the remaining will consist of 6 active and 2 placebos. For cohort 5, the numbers will consist of 6 active and 2 placebos.

12.3 Analysis Sets

Two sets of populations for analysis are distinguished, the Safety Set and the PK Set. Table 10 shows the analyses scheduled at minimum for each analysis set.

Table 10: Overview on the analyses scheduled at minimum for each analysis set

Analysis	Safety	PK
Demography/baseline characteristics	X	
Safety	X	
PK		X

Subjects are assigned to analysis sets prior to database closure as follows:

12.3.1 Safety Set

The Safety Analysis Set includes data from all enrolled subjects receiving any amount of IP. However, the data from each cohort will be summarized separately. No imputation for missing data will be made.

12.3.2 PK Analysis Set

The PK Analysis Set will include data from subjects who were treated and have no missing data affecting the PK assessment. No imputation for missing data will be made.

12.4 General Statistical Considerations

The statistical analysis aims at descriptive analysis of the study data by cohort or study phase. No pre-formulated hypothesis is tested, but significance tests may be applied in an exploratory fashion. Both raw data and changes since baseline will be assessed.

Details of the statistical analysis will be summarized in a separate statistical analysis plan (SAP). Changes from the statistical analysis outlined here will be detailed and justified in the SAP or in the final report, although additional exploratory analyses may be included without being considered a change from the protocol.

Continuous variables will be summarized by cohort and phase at each visit in tables and will include the number of subjects, mean (μ), standard deviation (SD), median, minimum (Min), and maximum (Max). Categorical variables will be presented by cohort at each visit in tables as frequencies and percentages. In general, for each parameter, both the raw value at each visit and its change or percent change from baseline will be presented. Tests for significance of differences may be used to detect differences between cohorts or treatment groups in a purely exploratory fashion. Due to the small sample size, Fisher's Exact Test will be used for categorical data [40]. For metric data, T Test, Mann Whitney Test, Anova Test and Kruskal-Wallis Test will be used as appropriate, if applicable followed by appropriate *post hoc* tests. The paired T Test or the Wilcoxon Test will be used to test changes between assessments. Graphs will be generated as appropriate.

Listings of individual subjects' data will be produced.

12.5 Subject Disposition, Demography and Baseline Characteristics

Enrollment, protocol deviations, and discontinuations from the study drug and the study will be summarized by phase and cohort in the safety population. Screening failures are not evaluated in detail but tabulated by number and by the applicable eligibility criterion. The number and percentage of subjects in each analysis population will be provided. A listing will show the reasons for exclusion from each analysis population.

Demographic data, medical history and baseline characteristics will be descriptively summarized by cohort in the safety population. For each cohort and for the total study population, these data are compared between the treatment groups in order to ensure comparability.

12.6 Safety Analysis

All safety analyses will be conducted using the Safety Analysis Set and will be presented by treatment group and cohort. Furthermore, all safety findings are itemized and compared by treatment group. AE and SAE data will be presented and tabulated according to MedDRA classification (see section 13.7). AEs will be summarized by the number of subjects reporting the events and by System Organ Class (SOC) and Preferred Term (PT), by seriousness and severity, by outcome and by relationship to IP.

The lung function measurements (TLC, DCO, FEV1, FeNO, CPET) and blood O_2 saturation will be presented in tables and graphically per cohort and time point. Laboratory (urinalysis, serum chemistry and hematology) parameters and vital signs will be tabulated by visit using descriptive statistics. The value at each visit as well as the change from baseline will be presented. ECG data and the findings of the physical examination and of imaging techniques will be reported as listings with abnormal and clinically relevant findings identified and summarized in tables. Numerical data are summarized as applicable per cohort.

12.7 PK Analysis

All pharmacokinetic analyses will be conducted using the PK Analysis Set. Non-compartmental pharmacokinetic parameters will be reported as geometric mean, %CV, minimum and maximum including: C_{max}, T_{max} and AUC.

Finally, the concentration will be plotted vs. nominal time for the PK profile both for individual subjects and as mean concentration per cohort.

12.8 Handling of Missing Data

All data will be analyzed as recorded. No imputation of missing data is foreseen.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Ethical Conduct of the Study

This study is conducted in accordance with this protocol, Good Clinical Practice (GCP [11]), the basic principles underlying the Declaration of Helsinki [41], all applicable legal and regulatory requirements and all applicable requirements possibly made by the Independent Ethics Committee (IEC) involved. All participants will be insured.

13.2 Independent Ethics Committee

In accordance with GCP and the applicable legislation and regulation, all relevant material for the conduct of this study is reviewed and approved by the responsible IEC. This material may include, but is not limited to:

- The final and fully signed version of this protocol
- A description of recruitment arrangements
- Healthy volunteer information and the informed consent procedure
- A justification of the suitability of the investigator and the trial site
- A confirmation of the subjects' insurance, including the indemnity
- A documentation of financial arrangements (will be in a protocol addendum)

The IEC is informed of all subsequent protocol amendments or new versions of any of the aforementioned documents. Reporting of SAEs to the IEC is described in section 11.2. Study duration is not expected to exceed one year; therefore, no prolongation of IEC votes is required.

13.3 Establishment of an SMC

A Safety Monitoring Committee (SMC) will be established to review and evaluate safety information including Aes (even if the relationship to the IP is unlikely) as well as PK data. The SMC will consult additional experts if necessary. Additional non-voting members may participate in the meetings.

The SMC will be consulted at the following occasions:

- Initial meeting
- At the end of treatment of each cohort. For the conduct of the SMC meeting, it is not necessary that data are already clean, but reasonable efforts will be made to provide the best data quality possible to the SMC.
- Upon reporting of severe Aes or Aes of any intensity, where a causal relationship to the medication cannot be ruled out.
- At investigator request
- If the study team becomes aware of any information from the literature or other trials that the participants of this study are exposed to an undue risk.

Recommendations of the SMC may include: (1) discontinuation of the trial; (2) interruption of the trial, (3) dose changes (reductions), (4) adjustment of the schedule for PK sampling, (5) additional tests or other recommendations to improve the safety and well-being of the

subjects. Sponsor's medical representative and the Principal Investigator will both have the right to veto recommendations in the SMC.

13.4 Informed Consent

Documented informed consent is a prerequisite for any study related procedures. Interested potential subjects are informed about the study, the procedures, and the associated risks in person and in writing by using the IEC-approved subject information and informed consent form. After discussion and covering any questions the subject may have, and after sufficient time for consideration, the subject is offered to date and sign the ICF together with the investigator. Signed ICFs are stored in the investigator site file and are made available for verification by monitors or authorized regulatory representatives at any time. A copy of the signed ICF is handed out to the subject. The consenting process including any questions arising during this is documented in the subject's source data.

If a protocol amendment is released or information becomes available that might affect the subject's willingness to continue the trial, especially information regarding the subject's safety, the subject must re-consent by signing a new version of the ICF before study related procedures are continued. The new version of the ICF has to be approved by the responsible IEC. The procedure for signing the new ICF is the same as for the original version at enrollment.

13.5 Subject Confidentiality and Data Protection

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted in the ICF/Subject information is prohibited. During the study, person- and health-related data are collected and analyzed. The risk of unintended disclosure of these data is mitigated by pseudonymization and restricted access.

- Pseudonymization means that each subject is identified only by means of a unique number (ID) in the frame of study-related data collection, including but not restricted to the CRF and SAE reports. The ID consist of a two-letter country code, a two-digit site and a three-digit subject number, e.g. "XX-YY-ZZZ". ID and subject identity are only linked in subject identification log which is kept strictly confidential by the investigator.
- Restricted access means that all study records kept at the trial site are kept confidential in appropriate and safe storage facilities [11] In the frame of this study, the European General Data Protection Regulation is strictly observed and prescribes the procedure for confidentiality breaches. The ICF (section 13.4) informs subjects about the processing of their personal and health-related data and about their respective rights.

In order to ensure compliance with applicable regulation and legislation, data generated in the frame of this study including source data must be available for inspection upon request by competent authorities.

13.6 Case Report Forms and Study Records

13.6.1 Source Data

Source documents are defined as hand-written, printed, optical or electronic documents containing source data (e.g. hospital records, laboratory notes, device accountability records, photographic negatives, radiographs, records kept at the investigation site, at the laboratories and at the medico-technical departments involved in the clinical investigation).

All data reported in this study, have to be correct, accurate and completely reflect the content of the source data. The investigator must permit study-related monitoring, audits, IEC review and inspections by competent authorities. Therefore, he would provide access to the source data which is transcribed to the CRF for the study, e.g. general practice charts, hospital notes, appointment books, original laboratory records, to the sponsor, authorized representatives of the sponsor such as monitors and auditors, and competent regulatory authorities.

For laboratory data, including safety laboratory, the laboratory printouts are defined as the source data. In case of X-rays, CT scans, and ECGs, the images and reports are the source data. The investigator's assessment of abnormal values or findings is documented, dated, and initialized on these printouts, or on the X-ray images, if these are present as prints. Relevant laboratory or radiological data are transferred to the CRF.

Any party with access to study records shall take all reasonable precautions consistent with applicable regulatory requirements to maintain the confidentiality of subject identifying information and sponsor's confidential and/or proprietary information.

13.6.2 CRF

Clinical data will be collected using a case report form (CRF). The CRF is available for inspection and review by applicable regulatory authorities, the IEC, and auditors or other designees authorized by UNION therapeutics.

In the CRF, subjects are identified by their ID; this number does not contain subject initials, exact birth dates or other information which would facilitate the identification of specific individuals. Recording of age or year of birth is permissible, but not of the day of the birth. Subject confidentiality is further specified in section 13.5.

13.6.3 Handling of Data Outside the CRF

Some study data are handled outside the CRF, including:

- Pharmacokinetic data (section 6.2.3)
- Classification of PDs (section 13.8)
- Assignment of subjects to analysis sets (section 12.3)
- Coding of Aes, medical history, and concomitant medication (section 13.7)

All these data are integrated into a separate database which is fused with the clinical database

13.6.4 Data Management

Completed CRFs are transcribed to the study database. Completeness and plausibility of the eCRF entries are ensured by a review by the DM. The foreseen checks and their documentation are described in the Data Management Plan (DMP).

At the end of the study, the following requirements apply for the locking process of the study database:

- All data have been entered and monitored
- All manual checks have been conducted and all queries solved, or it has been determined that specific queries cannot be solved
- All CRF pages have been signed by the investigator
- Coding has been completed according to section 13.7 and approved by the sponsor
- PDs have been classified as “major” or “minor” (section 13.8) and approved by the sponsor
- Subjects have been assigned to analysis sets (section 12.3) and approved by the sponsor
- The sponsor and the DM both agree in writing to perform the database lock

Data management procedures are described in more detail in a separate DMP.

13.7 Coding

Adverse events and medical history will be coded using the Medical Dictionary of Regulatory Activities (MedDRA) on the levels of “preferred term” (PT) and “system organ class” (SOC). In order to enable a timely generation of queries concerning ambiguous original terms and to support the early detection of safety signals, coding will be carried out on a continuous basis as soon as the respective data have been monitored. Therefore, the most recent version of MedDRA available at first-subject-first visit will be used.

Concomitant medication is coded by using the Anatomical Therapeutic Chemical Classification System (ATC), levels 2 and 5. Results of the coding process are reviewed by the sponsor or an authorized representative prior to database closure. The DMP will contain additional specifications concerning coding.

13.8 Protocol Deviations

PDs are defined as variations from the protocol. A major deviation is a PD that affects the safety or the rights of the subject or confounds interpretation of data. All other deviations, e.g., omitted investigations etc., are considered as minor. PDs are recorded and explained in the Monitoring Visit Report The classification of PDs as “major” or “minor” is reviewed by the sponsor or an authorized representative prior to database closure.

13.9 Data Quality Assurance

Accuracy, correctness, and completeness of all data entered to the CRF is checked through a variety of strategies:

- Monitoring as outlined in section 13.11.1 and specified in detail in the monitoring plan
- All CRF entries are reviewed by the DM according to a predefined manual check list which is part of the DMP.

Familiarity of all personnel using the CRF is ensured by appropriate training.

13.10 Investigator Requirements

Each investigator must adhere to GCP [11], all applicable legal and regulatory requirements and to the most recent version of this protocol. The investigator must obtain written approval of any changes to the protocol from UNION therapeutics prior to seeking approval from the responsible IEC, for any changes to the protocol. It is the responsibility of the investigator to enroll only subjects who meet all eligibility criteria and for whom a correctly filled, signed, and dated ICF is available.

13.11 Sponsor Requirements

13.11.1 Study Monitoring

UNION therapeutics ensures proper monitoring of the study with special attention to verification of all clinical requirements, the completeness, consistency and accuracy of the data, adherence to protocol, GCP [11] and compliance with applicable laws and regulations. Authorized, qualified representatives of the sponsor contact and visit the investigational site at regular intervals to verify the adherence to the study protocol and local legal requirements, to perform source data verification and to support the investigator in his study related activities. They will be allowed, on request, to review the various records relevant to the trial, i.e. the CRF and other pertinent data, provided that subject confidentiality is maintained in accordance with local requirements.

The following visits are planned: site initiation, routine monitoring visits, remote monitoring, and close-out visit. More details are given in a separate monitoring plan.

The investigators agree to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved in a timely manner.

13.11.2 Audits and Inspections

UNION therapeutics or its authorized representatives may perform audits at any time during or after completion of the clinical study. The investigator will provide any study-related documentation to the auditor, the study center personnel will be available to answer any questions, and the auditor will be able to inspect facilities and records relevant to this study. The same applies to inspections conducted by competent authorities.

13.12 End of Study and Retention of Data

After completion of the study, i.e., after the last visit of the last subject, a clinical study report according to ICH E3 [42] and ICH E9 [43] including all statistical analyses is submitted within twelve months.

The investigator will retain records and documents pertaining to the conduct of this study including CRF data as photo or carbon copy, source documents, ICFs, laboratory results, and IP logs for at least 25 years. If the No study records shall be destroyed without prior authorization from UNION therapeutics.

13.13 Protocol Amendments

This is an Ascending Dose study aimed at assessing safety of increasing doses. If symptoms of toxicity are found in the first cohort(s), the doses foreseen for the subsequent cohort(s) may be adjusted (the maximum dose to be tested will not exceed 6 mL).

Any major changes in the conduct of the study are documented in a new version of this protocol, or in a protocol amendment. Previous versions of this protocol are archived, changes and their underlying rational are summarized in the version history (section 15) of each new version. Minor modifications concerning the statistical analysis or data management will be described and justified in the SAP or DMP, respectively.

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15 VERSION HISTORY

Version	Summary of Changes
V1.0	Generation of new document.

16 APPENDICES

16.1 Appendix A: Key Personnel

Sponsor:	UNION therapeutics A/S Tuborg Havnevej 18 2900 Hellerup, Denmark
Project Manager	Mads Jellingsø (UNION therapeutics A/S) Tuborg Havnevej 18 2900 Hellerup, Denmark
Chief Medical Officer	Philippe Andres MD (UNION therapeutics A/S) Tuborg Havnevej 18
NCI	Prof. Vibeke Backer, MD DMSci Center for Physical activity Research, CFAS, Rigshospitalet Ole Maaløes vej 24, entrance 75 2200 København N, Denmark
PI	Jesper Sonne, MD, DMSci Study Center: DanTrials ApS, Bispebjerg og Frederiksberg Hospital Zelo Phase I Unit Indgang 11B, 1. Sal, Nielsine Nielsens Vej 6B 2400 København NV, Denmark

Clinical Research Organizations:	Phase 1 Clinical Unit (Zelo Fase I Enhed): Torben Balchen Director DanTrials ApS c/o Bispebjerg og Frederiksberg Hospital Indgang 11B Nielsine Nielsens Vej 6B
Pharmacovigilance	
Data Management and Statistics	
Medical Writer	

Local Laboratories	Department of Clinical Biochemistry Bispebjerg og Frederiksberg Hospital Indgang 80B, 1. sal Nielsine Nielsens Vej 4B 2400 København NV, Denmark
Laboratory for SARS-Co-V-2 and Microbiome Analysis	Klinisk mikrobiologisk afdeling, afsnit 9301 Rigshospitalet Henrik Harpestrengs Vej 4A 2100 København Ø
Central PK Laboratory	

16.2 Appendix B: Inhalation Device ( ) and Nasal Spray Device

Figure 2, Figure 3 and Figure 4 show some selected properties of the Nebulizer System,

Instructions for use for both devices are specified in a separate Instruction Manual.

16.3 Appendix C: Laboratory Parameters

Table 11: Laboratory data collected in the frame of safety assessment

Clinical Chemistry	Hematology	Urinalysis (Dipstick)
Total Bilirubin	Erythrocytes	Glucose
Calcium	Hematocrit	Bilirubin
Blood Urea Nitrogen	Mean corpuscular volume	Ketones
Creatinine	Hemoglobin	Specific gravity
Glucose	Mean corpuscular hemoglobin	Blood
Potassium	Mean corpuscular hemoglobin concentration	pH
Magnesium	White blood cell count	Protein
Sodium	Basophils*	Urobilinogen
Phosphorus	Eosinophils*	Nitrite
Albumin	Lymphocytes*	Leucocytes
Total protein	Monocytes*	
Alanine aminotransferase	Neutrophils*	
Aspartate aminotransferase	Platelets	
Alkaline phosphatase	Partial thromboplastin time	
Creatine phosphokinase	International normalized ratio	

* Differential Count