APPENDIX II INTERVENTION SPECIFIC APPENDICES

D. INVESTIGATIONAL PRODUCT: LTX-109 nasal spray 0.5% weight/weight (w/w) and placebo (0% LTX-109).

1. INTRODUCTION, RATIONALE AND PROTOCOL STRUCTURE

Many respiratory viruses, such as SARS-CoV-2, Influenza and Respiratory Syncytial Virus (RSV), primarily spread from person-to-person through aerosol or droplets that enter the body through orifices, such as the nose [1,2]. The nose is described as the main site for initial virus replication prior to virus migration towards the lower respiratory tract, which usually takes place 5-7 days after symptom onset [3-5]. Virucidal interventions administered intranasally early in the course of the disease could therefore be highly effective in decreasing viral load and limiting viral spread. Given that the nose is an important portal for host cell entry, interventions that can reduce the nasal viral load, and spread to the lower airways and beyond have the potential to ameliorate, or avert, serious or systemic infection.

LTX-109 is a synthetic peptidomimetic with antimicrobial and antiviral properties capable of lysing the bacterial membrane and the viral envelope. LTX-109 is expected to eradicate or at least significantly reduce the viral load in the nose and nasopharynx by a direct virucidal effect. LTX-109 was confirmed virucidal *in vitro* against four enveloped viruses, SARS-CoV-2, Influenza A, Influenza B and RSV, and has demonstrated antimicrobial activity *in vitro* against a broad range of bacteria, fungi and yeast [6]. *In vivo* studies have demonstrated virucidal action of LTX-109 nasal spray administered prophylactically in preventing SARS-CoV-2 infection in a hamster model [7]. More importantly, LTX-109 nasal spray has demonstrated effectiveness in treating SARS-CoV-2 and Influenza infections in hamster [7] and ferret [8] models, respectively.

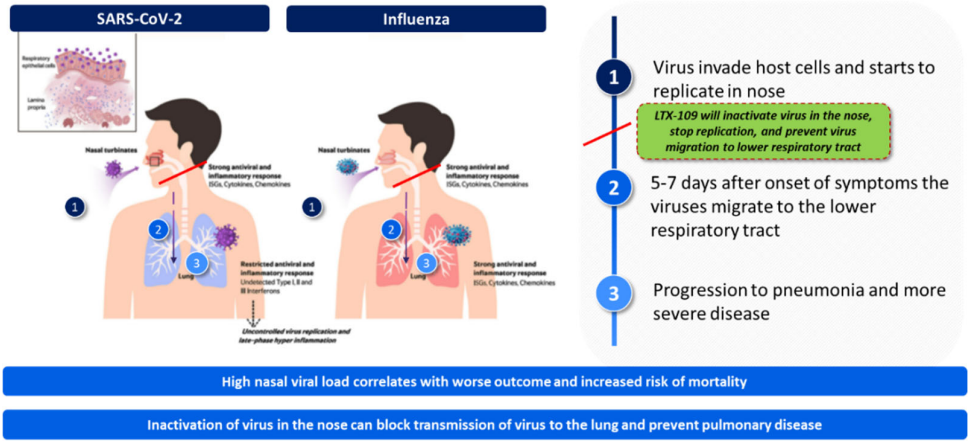

Figure 1. Treatment principle.

Treatment and clinical trial considerations

LTX-109 has been shown safe and tolerable with negligible systemic uptake in 8 clinical studies in humans mainly focusing on topical (skin) application and intranasal gel administration in multiple dosages and days of treatment [9]. LTX-109 showed no genotoxic potential, concluded from studies showing no structural chromosomal aberrations [10], no mutagenic activity [11] and no inducement of micronuclei [12]. However, since reproductive toxicology data is not yet available, women that are currently pregnant or breastfeeding were and will be excluded from participation in studies with LTX-109, and measures preventing a pregnancy in female participants during trial participation will be implemented.

The overall aim of the current study is to test the efficacy of the LTX-109 nasal spray for treatment of respiratory infections (COVID-19 or Covid-like-illness) in patients presenting to their General Practitioner's (GP) practice, or primary health care service (e.g. pharmacy). Treatment with the LTX-109 nasal spray will be initiated within 2 days since onset of symptoms to ensure that viral migration has not yet taken place. If effective, LTX-109 nasal spray could reduce viral load and potentially limit viral migration into the lower respiratory tract, ultimately resulting in improved recovery from respiratory infections.

The 0.5% w/w LTX-109 nasal spray formulation, to be tested in this study, has been developed for prophylactic use and treatment of respiratory infections. The safety and tolerability profiles of the LTX-109 intranasal spray in humans are expected to be similar to the safety and tolerability profiles of the gel formulation. Systemic absorption and thereby exposure is expected to remain very low [13].

This LTX-109 study will follow the principles described in ECRAID-Prime's master protocol for a phase II study and will focus on a proxy for efficacy, accelerating viral clearance of respiratory viruses, and will evaluate safety and tolerability throughout the study.

2. OBJECTIVES

2.1 Primary objectives

Viral clearance by LTX-109 nasal spray (0.5% w/w) measured at Day 3 after inclusion as compared to placebo nasal spray (0% LTX-109).

2.2. Secondary objectives

Additional main secondary objectives, to ECRAID-Prime's master protocol, include:

- Viral load reduction from Day 0 (baseline) to Day 1, to Day 3 and to Day 5;
- Viral clearance at Day 1 and Day 5.

3. STUDY DESIGN

3.1 Study Design

In this phase II study, LTX-109 (0.5% w/w) nasal spray will be tested under ECRAID-Prime's master protocol. Participants will be randomised in a double-blind fashion to LTX-109 (0.5% w/w) or placebo (vehicle, 0% LTX-109). Patients need to be randomised and IMP intake initiated within 2 days since onset of symptoms of a respiratory tract infection.

3.2 Study Description

As per ECRAID-Prime's master protocol, with the following changes for LTX-109 nasal spray evaluation:

- Randomisation and IMP intake initiation within *2 days* since onset of symptoms;
- IMP administration 3 times a day for 3 consecutive days (starting at Day 0 -first dose after the baseline swab- with 3 or 2 doses -depending on moment of inclusion-, and ending on Day 2, or Day 3 with 1 dose);
- Swabs will be taken at Day 0 (baseline), Day 1, Day 3, and Day 5. If feasible, swabs will be taken by a health care professional, otherwise by self-swabbing;
- Baseline CRF and 28-days diary adapted to the phase II study LTX-109 IMP evaluation, with tolerability assessment for 3 days, and AE assessment for 7 days;
- Study duration is 28 days.

3.3 Schematic diagram of study design

The study will have the below depicted scheme.

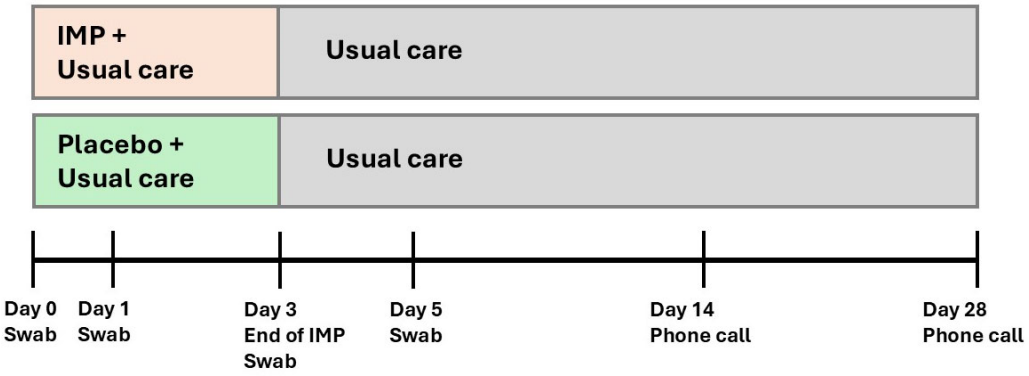

Figure 2. Study scheme.
IMP: Investigational medicinal product.

3.4 Duration of the study per participant

Duration of the study is 28 days (no long-term follow-up). IMP self-administration of a total of 9 doses over 3, or 4 days, depending on the moment of the first dose.

3.5 Nested qualitative study

As per ECRAID-Prime's master protocol.

4. STUDY POPULATION

4.1 Population (base)

As per ECRAID-Prime's master protocol.

4.2 Inclusion criteria

As per ECRAID-Prime's master protocol. Changed and additional inclusion criteria for LTX-109 nasal spray evaluation:

- Inclusion within 2 days since onset of symptoms;
- For women of child-bearing potential (WOCBP)*: prepared to use a highly effective method of contraception* or abstinence from heterosexual intercourse* for 30 days before and after terminating study medication intake, and a negative pregnancy test on day of inclusion.

* See master protocol.

4.3 Exclusion criteria

As per ECRAID-Prime's master protocol. Additional exclusion criteria for LTX-109 nasal spray evaluation:

- Known to be currently pregnant or breastfeeding;
- Current or history of moderate to severe epistaxis or hereditary hemorrhagic telangiectasia;
- History of cerebral spinal fluid leaks via the sinuses/nose;
- Recent nasal fracture, nasal tumors, nasal masses, meningoencephalocele, and/or nasal surgery within the previous 2 weeks;
- Any known nasal anatomical anomalies.

4.4 Sample size calculation

Based on a 20% difference in viral clearance between groups at Day 3 (10% in the placebo group and 30% in the LTX-109 (0.5% w/w) group), 56 patients in the LTX-109 (0.5% w/w) arm and 56 patients in the placebo arm are needed. This has a power of 80% based on a one-sided Fisher's exact test with level of significance of 0.05. Taking into account that at baseline the swab of 30% of participants does not show the presence of a virus and 10% of participants do not provide the Day 3 swab, the number needed per arm is 94, 188 in total.
The sample size was computed using PASS 2024, version 24.0.3.

5. STUDY TREATMENTS

5.1 Investigational Products

5.1.1 Name and description of the IMP

LTX-109 (International Nonproprietary Name: Voxvoganan) nasal spray: 0.5% w/w and placebo (vehicle, 0% LTX-109) nasal spray.
LTX-109 (0.5% w/w) and placebo nasal sprays will be supplied by Pharma Holdings AS, Killengreens gate 8, 9008 Tromso, Norway. Nasal sprays will be manufactured by HWI Group GmbH, Frankfurt, Germany. Labeling and distribution will be performed by Myonex GmbH, Berlin, Germany.

5.1.2 Status of development of the IMP

LTX-109 has completed the required preclinical studies and was used in 8 clinical trials in humans in the gel formulation [9]. Results of key *in vitro* and *in vivo* studies are summarized below. Scientific advice from EMA has supported evaluation of LTX-109 as a nasal spray in patients with respiratory infections in a clinical study and EMA recommendations have been taken into account when preparing for and designing the current study [14].

5.1.3 Study data on LTX-109, in vitro and in vivo

SARS-CoV-2

An EU-funded (ISIDORe) study to obtain *in vitro* and *in vivo* data on therapeutic and prophylactic efficacy of LTX-109 towards SARS-CoV-2 was completed in 2024 [7]. The *in vitro* study allowed definition of the Half Maximal Inhibitory Concentrations (IC_{50}) and the Full Maximal Inhibitory Concentration ($IC_{99.9}$) of LTX-109 towards SARS-CoV-2 upon 10-minute exposure to LTX-109, see Figure 3.

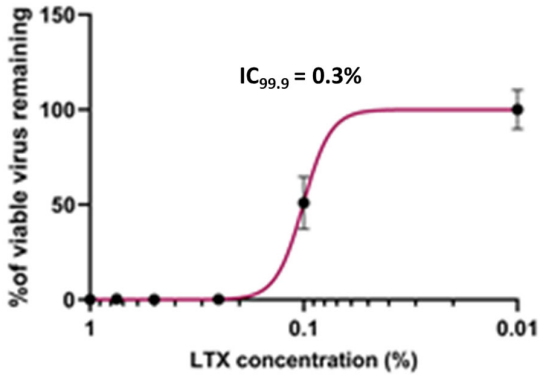

Figure 3. Infectivity of treated samples expressed as percentage to untreated control.
A non-linear regression fit curve was calculated to infer the IC50 and the IC99.9 of LTX-109 at 10 minutes exposure.

The *in vivo* prophylactic study used a hamster model. LTX-109 nasal spray formulations 0% (vehicle-treated control group) or 3% were administered intranasally according to the following schedule: 20 minutes before SARS-CoV-2 inoculation and then 4, 5.5, 8 and 10 hours post-infection. The data demonstrated that pre- and post-exposure treatment with LTX-109 prevented replication of the virus in the nose and its further spread through the respiratory tract, irrespective of the challenge dose: 10^0 or 10^1 focus forming units/ml (FFU/ml). Unlike the control groups in which the viral genome was detected in oral swabs from 24 and up to 72 hours post-inoculation, oral swabs collected from hamsters in the treated groups resulted in negative swabs at all timepoints, as depicted in Figure 4.

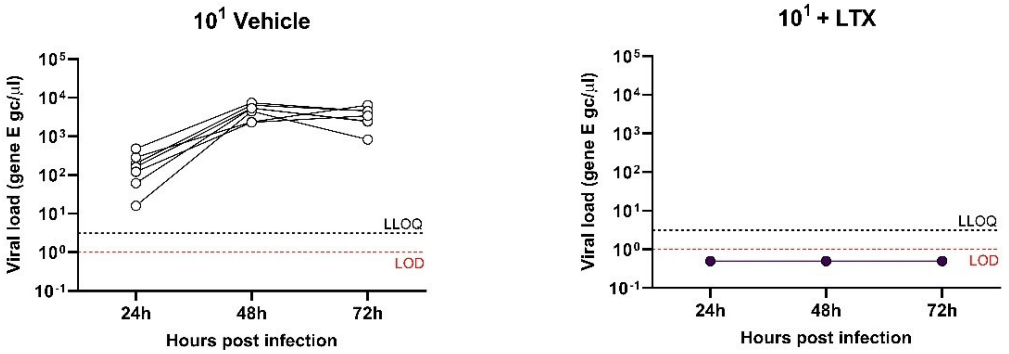

Figure 4. Viral loads in oral swabs from animals challenged with 10^1 FFU and treated with 0% or 3% LTX-109.
Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E-gene. Individual values are shown. The dashed lines indicate Level of Detection (LOD, in red) and Lower Level of Quantification (LLOQ in black) values. No viral replication was recorded in the LTX-109 treated group.

The nasal wash samples showed no replication competent virus for animals treated with LTX-109 3%, while all samples from the vehicle-treated animals did contain replication competent virus. Likewise, replication competent virus was present in samples from the lung while no replication competent virus was found in the lungs of the animals treated with the LXT-109 nasal spray, as depicted in Figure 5.

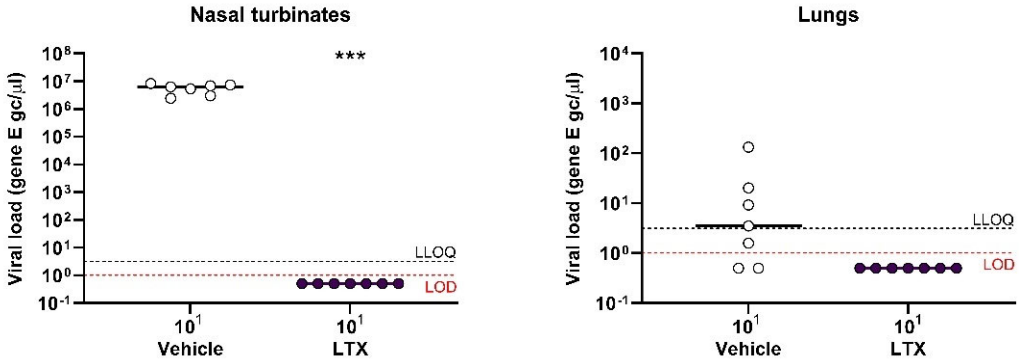

Figure 5. Viral loads in nasal turbinates and lungs from animals challenged with 10^1 FFU and treated with 0% or 3% LTX-109, collected 3 days after infection.
*Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E gene. Individual values are shown, with median values. The dashed lines indicate LOD (red) and LLOQ (black) values. No viral replication was recorded in the LTX-109 treated group. Statistics, non-parametric Mann-Whitney test, were calculated using Prism GraphPad (v.10.2). *** p<0.001.*

In a second study, on *in vivo* treatment efficacy, 21 hamsters were inoculated (under isoflurane gas anesthesia) intranasally with 10µL (5µL per nostril) of 10^1 FFU of SARS-CoV-2 BA.5 variant. The challenge dose was selected based on preliminary experiments aimed at determining the dose associated with 100% infection in hamsters of this age. Based on these experiments, the $100HID_{100}$ dose was selected for the treatment efficacy study. Animals were divided into 3 groups (n=7 per group) treated with vehicle, 1% or 3% LTX-109 intranasally according to the following time schedule: 4, 5.5, 8, 10, 22, 27, 32, 36 and 46 hours post-inoculation, as depicted in Figure 6.

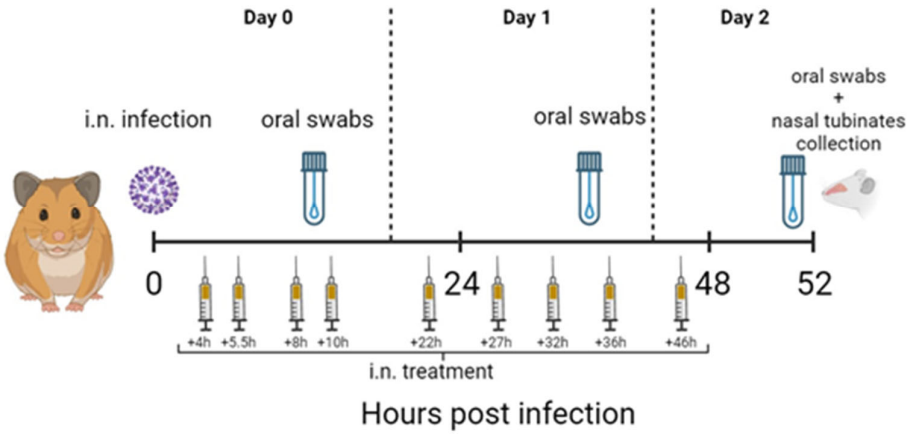

Figure 6. Graphical representation of the in vivo therapeutic study.

Quantitative real-time polymerase chain reaction (PCR) performed on oral swabs collected at different time points showed active replication of SARS-CoV-2 in all the animals (7/7) of the vehicle group (Figure 7) for which a mean load of 3.98±3.51 log_{10} genomic copies/µl was recorded 52 hours post-inoculation. In 2/7 hamsters treated with a 1% concentration of LTX-109, small amounts of genomic material were observed at 36 hours post-inoculation. However, similar loads were recorded for the same hamsters 24 hours later, suggesting that viral replication was minimal or abortive at this anatomical site. Overall, the 1% formulation significantly reduced the presence of the virus in the oropharynx ($p<0.05$ at 52 hours). With the 3% formulation, SARS-CoV-2 was not detected in any of the oral swabs throughout the study (Figure 7). Nasal turbinates were collected at the end of the study to evaluate viral load by quantitative real-time PCR and results show a clear dose-dependent effect of the treatments in reducing viral replication (Figure 8). In the vehicle group, hamsters reached a mean viral titer of 6.69±6.36 log_{10} genomic copies/µl. The highest reductions in viral load were obtained in the hamsters treated with the 3% formulation, achieving a statistically significant 320,000-fold ($10^{5.5}$) reduction in the mean viral load ($p<0.001$). The 1% LTX-109 formulation also significantly reduced the mean viral load by a factor of 1,300 ($10^{3.1}$) ($p<0.05$).

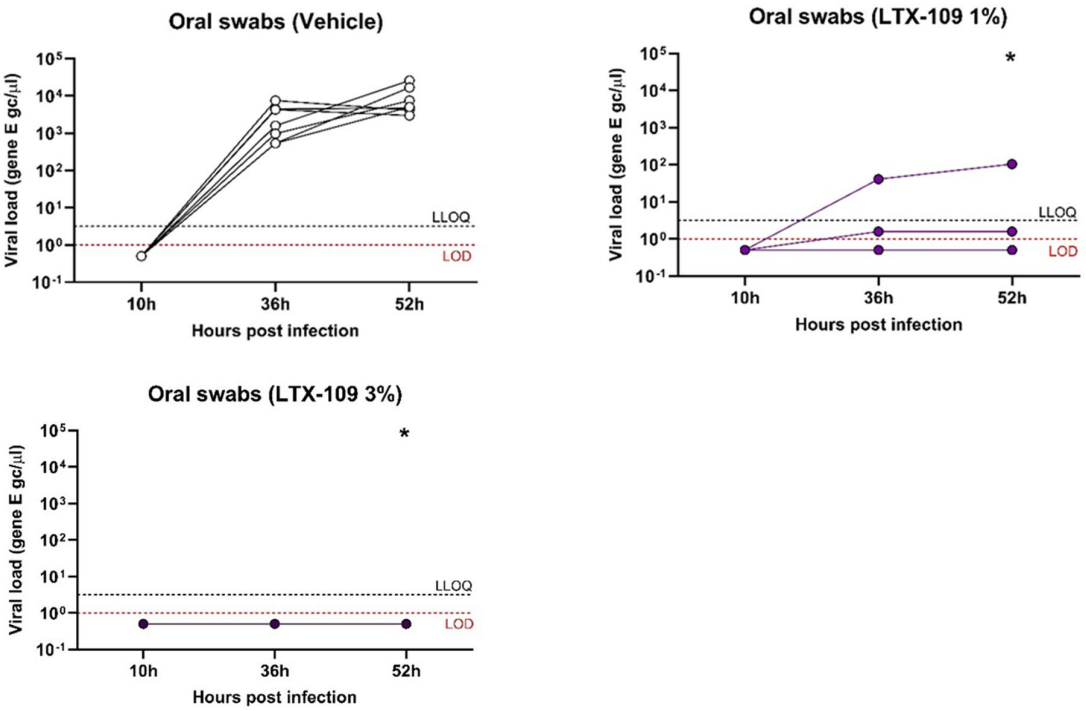

*Figure 7. Viral loads in oral swabs from animals challenged with 10^1 FFU and treated with 0%, 1% or 3% LTX-109. Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E gene. Individual values are shown. The dashed lines indicate LOD (red) and LLOQ (black) values. Statistics, a non-parametric 2-way ANOVA test with Dunnet's correction for multiple comparisons between vehicle and treated groups, were calculated using Prism GraphPad (v. 10.2). * p<0.05.*

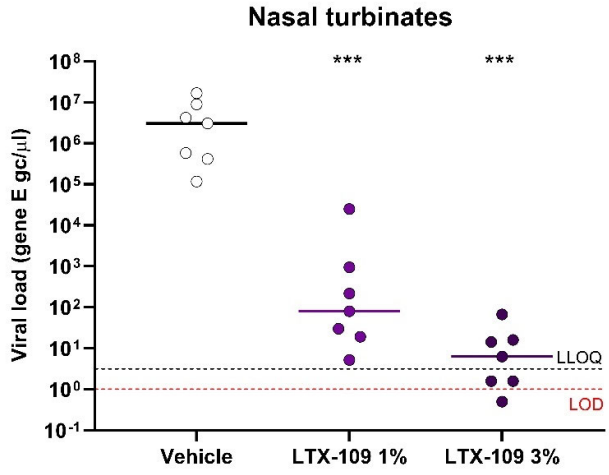

Figure 8. Viral loads in nasal turbinates from animals challenged with 10^1 FFU and treated with 0%, 1% or 3% LTX-109, collected 52 hours after infection.
*Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E gene. Individual values are shown, with median values. The dashed lines indicate the LOD (red) and LLOQ (black) values. Statistics, Mann-Whitney non-parametric t- test, were calculated using Prism GraphPad (v. 10.2). ***p<0.001.*

Animals treated with 3% LTX-109 exhibited extremely low viral loads; 1 out of 7 in this group was negative for SARS-CoV-2 and 2 animals had values below the LLOQ.

The treatment model was subsequently repeated with the exact same procedures for LTX-109 0%, 0.5% and 0.75% nasals spray formulations to explore the dose/effect relationship at lower concentrations (n=7 per group). The results are shown in Figure 9 and 10.
Results of the LTX-109 0.75% formulation were comparable to those of the 1% formulation. LTX-109 0.5% was slightly less effective but still exhibited a >3.5 Log_{10} or >99.9% reduction in viral load as compared to the nasal turbinate samples taken from vehicle treated hamsters.

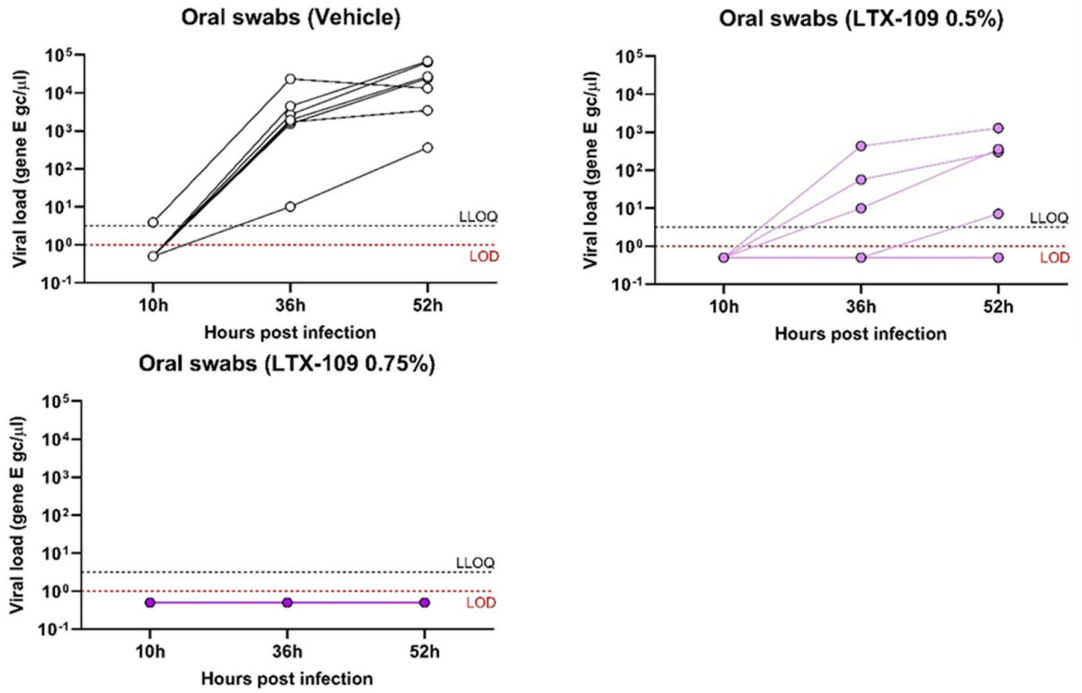

Figure 9. Viral loads in oral swabs from animals challenged with 10^1 FFU and treated with 0%, 0.5% or 0.75% LTX-109.
Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E gene. Individual values are shown. The dashed lines indicate LOD (red) and LLOQ (black) values.

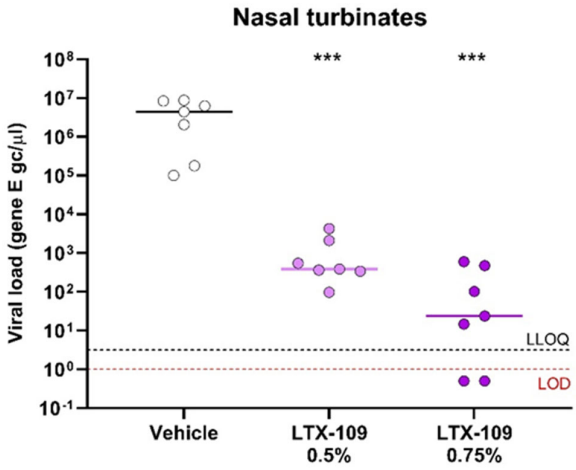

Figure 10. Viral loads in nasal turbinates from animals challenged with 10^1 FFU and treated with 0%, 0.5% or 0.75% LTX-109, collected 52 hours after infection.
*Viral load is expressed as genomic copies/µl of the SARS-CoV-2 E gene. Individual values are shown, with median values. The dashed lines indicate LOD (red) and LLOQ (black) values. Statistics, Mann-Whitney non-parametric t-test, were calculated using Prism GraphPad (v. 10.2). ***p<0.001.*

Influenza A and Influenza B

An *in vivo* study on LTX-109 efficacy on Influenza A in the commonly used ferret model for Influenza was completed in 2023 (*Mustela putorius furo*). The study was designed to investigate the antiviral efficacy of repeated intranasal administration of LTX-109 after inoculation with seasonal H3N2 Influenza virus via the intranasal route, compared to a vehicle treated control group.

Three groups of 6 animals and one group of 5 animals were intranasally inoculated with H3N2 virus on day 0. Following inoculation, LTX-109 was administered a total of 9 times during days 0 (4 times), 1 (4 times) and 2 (once before euthanizing). LTX-109 0%, 1%, 3% and 5% formulations were used. Ferrets were swabbed at 4 time points on day 0 and day 1, and once on day 2. Both virology and pathology in respiratory tissues and fluids were investigated. On day 2, three hours after treatment, bronchoalveolar lavage (BAL) was collected from all animals at the time of euthanasia. Samples were analyzed using an influenza A matrix-specific TaqMan real-time PCR for RNA and by cultivation to detect replication competent virus. None of the ferrets treated with the LTX-109 1%, 3% and 5% formulations had replication competent virus in their nasal turbinate samples while all samples from the vehicle treated group contained replication competent virus. Results from the real-time PCR and titration of replication competent virus (by cultivation) of the nasal wash and BAL samples are shown in Figure 11. In conclusion, ferrets treated with the LTX-109 1%, 3% and 5% formulations had a reduction in viral load compared to the vehicle treated groups, and complete eradication of replication competent virus. In addition, the LTX-109 1%, 3% and 5% formulations successfully prevented viral migration from the nasopharynx to the lungs.

In vitro, the IC_{99} for Influenza B has recently been determined at 0.15 % [15]. No *in vivo* data are available for Influenza B.

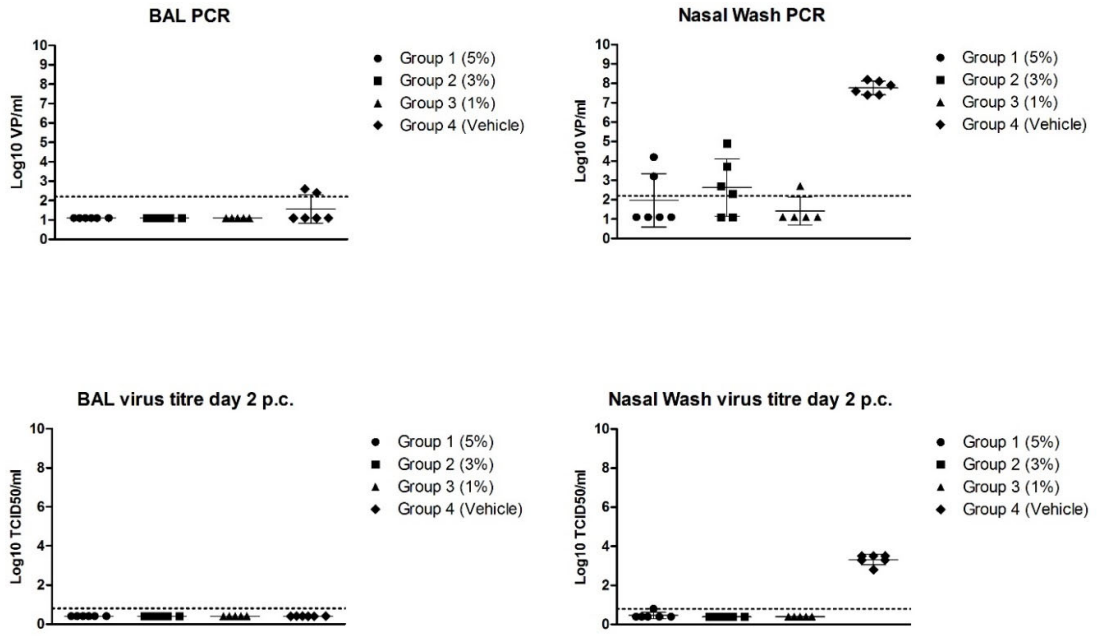

Figure 11. PCR and virus titer (VT) of BAL and nasal wash samples collected on day 2.
Individual values are shown by group, with group means ±SD shown by the solid black lines. The lower limit of detection (LLOD) for the PCR and for the VT are 2.2 Log^{10} virus particles/ml and 0.8 Log^{10} 50%-tissue culture infectious dose (TCID50)/ml, respectively, as indicated by the dashed lines. For graphical representation, samples having values lower than the LLOD are replaced by half of the LLOD value.

RSV

In vitro efficacy has been determined upon 1 hour exposure to LTX-109 1% and 0.1% formulations, resulting in an over 3-log reduction in RSV infectivity as compared to vehicle, corresponding to an over 99.9% reduction [16]. No *in vivo* efficacy data are available for RSV.

5.1.4 Description and justification of dosage and route of administration

LTX-109 (0.5% w/w) nasal spray will be administered intranasally three times a day [1 spray each nostril, equivalent to 280 µL volume total per dose (2 sprays)] for a total of 9 doses. Nose spray should be administered 6-7 hours apart (upon awakening, mid-day and before going to bed). Depending on the moment of inclusion, participants can take 3 doses on Day 0 (morning recruitment), then ending treatment on Day 2, or 2 doses on Day 0 (afternoon recruitment), then ending treatment on Day 3 with 1 dose.

The selected LTX-109 concentration and dosing regimen have been determined based on efficacy and safety data from *in vitro* and *in vivo* studies as described earlier, summarized in the Investigators Brochure and available in the following reports:

1) Study reports on *in vitro* testing of LTX-109 against SARS-CoV-2, Influenza A (H1N1) and RSV at 60 seconds and 1 hour exposure at VRS (Virology Research Services Ltd., UK);
2) Reports on electron microscopy imaging to as mode of action on SARS-CoV-2 (Norwegian University of Science and Technology) and Lenti-virus-like particles (Oslo University Hospital, Norway);
3) Study report (Viroclinics/Cerba Research, the Netherlands) on *in vivo* efficacy of LTX-109 nasal spray for treatment of Influenza A infection in ferrets;
4) Study report on LTX-109 efficacy *in vitro* against SARS-CoV-2 and *in vivo* studies on the LTX-109 nasal spray as prophylaxis and treatment for SARS-CoV-2 infection in hamsters (IZSV, Italy);
5) Report from Combined Feasibility and Repeat Dose Study of LTX-109 by Intranasal Instillation in Rats;
6) Report from Good Laboratory Practice (GLP) Toxicology study in rats on 14-day repeat dosing via intranasal instillation.

LTX-109 was tested *in vivo* for toxicology and local tolerance in a 14-day repeat dose GLP study with 3 intranasal administrations per day [13]. A dose of 7.2 mg/kg/day (as 4x5.0 µL, 3 times daily) of 3% LTX-109 was considered to be the No Adverse Event Level (NOAEL). This dose represents a safety margin of >100x over the 0.5% dosing of 280 µL TID to be used in this study in terms of mg/kg, and >4.8x in terms of surface area.

Administration guidance will be described and shown in the guidance for participants:
Prime by pressing the spray head so that a spray plume appears (3 times before first use, then once before next use). Tilt the head forward. Gently insert the bottle tip just into one nostril. Press on the other side of their nose with one finger to close off the other nostril. Aim slightly away from the center of the nose, point in the direction of the ear, and pump one spray. Sniff gently through the nose while applying, not inhaling deeply. Exhale through the mouth. Ensure that the spray container is held approximately vertically while spraying. Repeat application in the second nostril (priming not needed for the second nostril).
First administration will be done during the recruitment visit.

5.2 Additional considerations for trials involving a medical device
Not applicable.

5.3 Preparation and labelling of the Investigational Medicinal Products
The IMP will be manufactured by HWI Group GmbH, Frankfurt, Germany. IMP will be packed, labelled and distributed by Myonex GmbH, Berlin, Germany in such a way that the double-blind design of the study is effectively maintained throughout the study. IMP will be supplied in a 10 mL bottle with spray head. IMP labels will contain all information required for regulatory (by country) and identification purposes.
Participants will be allocated to 0.5% w/w LTX-109 (Voxvoganan) nasal spray, or placebo (vehicle, 0% LTX-109) nasal spray according to a medication pack number provided by the randomization system. User instructions will be provided, with administration guidance for 9 doses in total, over 3 or 4 days.

English label:

{Backbone text (if applicable)}

FOR CLINICAL TRIAL USE ONLY
Protocol: ECRAID-Prime **EU Clinical Trial Registration no.: 2022-501707-27**
Investigator: _____

Medication number: XXXXXX
Lot/Batch Number: BBBB-BB

Participant ID: PRIME2-I___I___I I___I___I I___I___I___I___I

Content: 1 bottle of 10mL LTX-109 (Voxvoganan) 0.5% w/w nasal spray or 10mL placebo nasal spray, 1 dose is 280µl in 2 sprays, ~30 doses per bottle for intranasal use

Instructions for use: 3 times per day (1 spray per nostril), 3 days, see instructions for medication use

Storage conditions: between 2°C-25°C Expiry date/re-test date: MM/YYYY

Sponsor: UMC Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands
Telephone: +31 88 755 5555

KEEP OUT OF REACH OF CHILDREN Return packaging and unused medication

6. OTHER TREATMENTS AND RESTRICTIONS

6.1 Concomitant therapy

6.1.1 Permitted medication

Participants should continue to take their usual medications, those prescribed during the consultation (e.g. antibiotics) and can take symptomatic treatments for their respiratory infection (antipyretics, analgesia and antitussives) throughout the study period. Use of treatments for the respiratory infection will be recorded for the duration of the study.

6.1.2 Prohibited medication

No prohibited medication.

6.1.3 Escape medication

As per ECRAID-Prime's master protocol.

6.2 Lifestyle restrictions

6.2.1 Contraception measures

Pregnant and breastfeeding women are excluded. See in/exclusion criteria and definitions of contraception measures in ECRAID-Prime's master protocol and section 4.2.

6.2.2 Other requirements

There are no other requirements.

7. TRACEABILITY, STORAGE, ACCOUNTABILITY AND COMPLIANCE

LTX-109 nasal spray should be stored between 2°C-25°C. IMP should not be used after the expiry date printed on the label. IMP is collected by study personnel after treatment has finished. Patients will report in their diary how many doses they took every day. Bottles will be weighed to check whether opened and used.

8. METHODS

8.1 Study parameters

As per the phase II study, the diary questions and timing of outcome assessments were adjusted for the LTX-109 nasal spray evaluation, including tolerability questions, and AE reporting for 7 days. Long-term follow-up data collection (beyond 28 days) was deleted.

8.2 Randomisation, blinding and treatment allocation

Participants will be randomised in a double-blind fashion to LTX-109 (0.5% w/w) or placebo (vehicle, 0% LTX-109). As per ECRAID-Prime's master protocol, randomisation is per a rapid, secure, web-based randomisation system (ALEA) using non-deterministic minimisation, with a random element. The minimisation factors for the LTX-109 evaluation will be age (<55/≥55 years), time since onset of symptoms (≤1/2 days), and participating country.

8.3 Study procedures

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

1. Follow-up sampling on Day 1, Day 3 and Day 5;
2. IMP intake for 3 days;
3. Ruling out pregnancy by a negative urine pregnancy test for all women of child-bearing potential prior to randomisation;

8.4 Withdrawal of individual participant

As per ECRAID-Prime's master protocol.

8.5 Replacement of individual participants after withdrawal

As per ECRAID-Prime's master protocol.

8.6 Discontinuation of treatment of individual participants

As per ECRAID-Prime's master protocol.

In addition, a participant should discontinue treatment in case of excessive and persistent nasal irritation and/or pain and contact their GP. A physician should be consulted if symptoms persist.

8.7 Premature termination of the study
As per ECRAID-Prime's master protocol.

9. SAFETY REPORTING
9.1 Adverse events (AEs)
As per ECRAID-Prime's master protocol, captured for 7 days.

9.2 Recording of serious adverse events (SAEs)
As per ECRAID-Prime's master protocol, captured for 28 days.

9.3 Follow-up of a pregnancy whilst being randomised to IMP
As per ECRAID-Prime's master protocol, the investigator has to notify the Sponsor, immediately and no later than 48 hours after being made aware of a pregnancy, initiated during study participation, despite taken measures and precautions. Participants will be asked at Day 28, via their diary, whether they (WOCBP) have become pregnant. In situations where WOCBP have become pregnant within 28 days of study participation, unblinding will take place. If unblinding reveals that the participant did receive LTX-109 (0.5% w/w) nasal spray, the participant will be asked to sign and date an informed consent for following up the pregnancy until birth due to the unknown safety profile of LTX-109 when used during pregnancy.The initial pregnancy report should be reported in a detailed, written report, using the "Pregnancy Initial Notification Form" (in the Investigator Site File (ISF) and should specify estimated date of delivery, obstetrician contact and name of maternity hospital. The investigator has to follow-up the participant until the end of the pregnancy or its interruption and to notify the outcome to the sponsor using the "Pregnancy Follow-Up Form" (in the ISF).

Warning:

If the pregnancy outcome fulfills a seriousness criterium (e.g.: anomaly or birth defect, fetal death, voluntary or therapeutic interruption of pregnancy, miscarriage needed a hospitalisation), the investigator has to notify this to the Sponsor as an SAE.

All pregnancies should be recorded in in the Pregnancy form located in the ISF and sent to the Safety Department of CTCC.

10. STATISTICAL ANALYSIS
For the primary analysis, LTX-109 (0.5% w/w) nasal spray will be compared to placebo (vehicle, 0% LTX-109) nasal spray.

Interim analyses

Safety and tolerability will be continuously monitored by the medical monitor and periodically by the DSMB that is established for ECRAID-Prime. Composition of the DSMB and procedures are described in the DSMB Charter.

11. ETHICAL CONSIDERATIONS
As per ECRAID-Prime's master protocol.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION
As per ECRAID-Prime's master protocol.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

An important class of natural antimicrobial peptides are the cationic peptides, the class of molecules that inspired the design of the LTX-109 molecule. LTX-109 has two overshadowing physical properties: it is catatonically charged with 3 cationic sites (at physiological pH), and it is lipophilic (lipid soluble) particularly caused by the tri-tert-butyltryptophan (Tbt) residue and the phenethyl C-terminal modification. These two properties are important in considering its antimicrobial activity.

LTX-109 has been designed to selectively attack the bacterial cell membrane and viral envelopes. The peptide does so by first interacting with anionic structures in the membrane through electrostatic interactions between the cationic sites in the peptide with anionic structures including LPS (lipopolysaccharide in Gram negatives), TA (teichoic acids in Gram positives), and PS (polysaccharide) in the bacterial membrane [17]. This interaction results in a high concentration of the peptides close to the inner (lipid) part of the membrane, a situation working as a preamble to the second step of the mechanism where the lipophilic part of the peptide buries itself into the lipophilic interior of the cell membrane which finally will lead to membrane lysis.

The mode of action of LTX-109 on the encapsulated virus is regarded similar to the action on procaryotic cell membranes, it acts by lysing the membrane/envelope surrounding the virus particle. The membrane of encapsulated viruses originates from the membrane of the infected cell, hence the membranes of encapsulated viruses are of eucaryotic origin. The viral envelopes, however, differ from the eucaryotic membranes in at least two aspects. First, the lipid composition can be different, and second, the membrane asymmetry is often lost, or reversed [18]. The outside of the virus envelope is thereby regularly rich in anionic lipids, like PS, enabling the peptides to selectively bind to the virus envelopes through electrostatic attraction at concentrations below those required to cause a cytotoxic effect on eucaryotic membranes. Numerous negative patches originate mainly from negatively charged lipid domains in the viral envelope and negatively charged areas on the “stalks” of the spike (S) proteins [19]. As LTX-109 is a cationic and amphiphilic molecule, micelle formation in aqueous solutions is an important part of its electrostatic attraction and rapid mode of action. Electron microscopy imaging suggests that lysis of the envelope is responsible for the effect [20].

It is expected that antimicrobial peptides are selective towards (encapsulated) viruses over eucaryotic cells. The eucaryotic cell membrane facing the exterior is electrostatically neutral. It does not contain LPS or TA. Furthermore, the eucaryotic cell membrane is asymmetric regarding the phospholipids, and the outer membrane leaflet does not contain PS, as all PS in the eucaryotic cell membrane is located in the inner membrane leaflet [19]. The net result of the outwardly neutral eucaryotic membrane is the fact that no charge interaction between the antimicrobial peptide and the eucaryotic membrane can take place. The only interaction possible is the lipophilic side chain vs. the lipid interior of the membrane resembling the second step in the mode of action discussed above. The absence of the first mode of action step for eucaryotic cells can explain the selectivity observed for antimicrobial peptides. Bacteria are killed and viruses are inactivated at much lower peptide concentration than needed to affect eucaryotic cells.

b. Previous exposure of human beings

This is the first clinical trial with LTX-109 nasal spray formulation. However, LTX-109 in a gel formulation has been investigated in 8 clinical trials in humans. Three of these trials have used the gel for “nasal decolonization of Staphylococcus aureus” and strengths of up to 5% LTX-109 have been used and found to be safe and tolerable when applied to the anterior nares with multiple dosing for up to 3 days.

LTX-109 in a gel formulation was administered to the nares (multiple dosing) and nasal cavity (single dose) in the following trials, summarized in the IB section 5, with no significant adverse safety signals:

1. C10-109-02: Phase I/IIa study on nasal decolonization colonized with methicillin-resistant/-sensitive Staphylococcus aureus (MRSA/MSSA).
2. C201-109-06: Phase I/IIa study, LTX-109 gel for nasal decolonization.
3. C22-109-08: Phase IIa study, 3% LTX-109 gel for nasal decolonization.
4. C21-109-09: Effect of single dose of 3% LTX-109 gel on COVID-19.

c. Induction of the mechanism in animals and/or ex-vivo

Efficacy of LTX-109 nasal spray has been demonstrated in a ferret therapeutic model with strengths 1-5%, and in prophylactic and therapeutic hamster models in strengths 0.5%-3%, as described earlier.

d. Selectivity of the mechanism

As explained earlier, the viral envelope and eukaryotic cell membrane are not similar in composition with respect to the combination of phospholipids and other fatty acids and embedded macromolecules.

Viral envelopes are different from host cell membranes for various reasons: 1) They are acquired at host cell membranes, the plasma membrane, but also other membranes like internal cell membranes such as the nuclear membrane, endoplasmic reticulum, and Golgi complex during maturation of the virus by the “budding”-process; 2) The lipids of the viral envelope are derived directly from the cell, but the proteins in the envelope are virus-coded. Enveloped viruses have their own transmembrane proteins (such as the spike protein in coronaviruses) and exclude host proteins from the viral envelope; 3) They use specific parts of the cell membrane (e.g. the endosome system) to assemble the virus particle; and 4) They are likely to use a combination of phospholipids which is distinctive to that particular part of the host cell, rather than being representative of the host cell membrane as a whole.

LTX-109 has been designed to preferentially bind and interfere with negatively charged membrane/envelope components on microorganisms. To assess the cytotoxicity of LTX-109 against potential host cells a series of *in vitro* cytotoxicity experiments have been completed against Human Embryonic Kidney 293 cells (HEK-293), human Hepatoma cells (HepG2), Human Umbilical Vein Endothelial Cells (HUVEC), Normal Human Fetal Lung Fibroblast (MRC-5) and Normal Human Epidermal Keratinocyte (NHEK) [20]. Compared to reference substances, the cytotoxic potential of LTX-109 is similar to, and in sometimes lower than observed for, antimicrobial agents currently approved for systemic and local administration (e.g. daptomycin, mafenide). Cell proliferation data generated in these experiments did not identify unacceptable cytotoxic potential for LTX-109.

e. Analysis of potential effect

The potential effect is expected to be and result from inactivation of virus similarly as demonstrated in the *in vivo* ferret and hamster models. As demonstrated in both species viral

inactivation should lead to fewer virus particles reaching the lower respiratory tract leading to reductions in both illness duration and risk of lower respiratory tract infections and complications.

f. Pharmacokinetic considerations

Systemic absorption is expected to be negligible from nasal administration of LTX-109 in line with administration of the LTX-109 gel formulation to the nose. The highest individual peak plasma concentration (T_{max}) in any of the previous trials in humans, 117 ng/ml, was recorded in one participant in trial C21-109-06 on nasal decolonization of Staphylococcus aureus where the LTX-109 gel was administered frequently: 3% w/w gel applied 4 times in one day of treatment, with 2 hours between the 4 doses. No SAEs or toxic effects were observed. The 117 ng/ml is still within orders of magnitude below levels of concern, based on the NOAEL for systemic clinical signs in preclinical IV studies in rats [21]. Similarly, the 14-days intranasal GLP toxicology study in rats with LTX-109 3% nasal spray formulation, resulted in a maximum absorption level of 112 ng/ml, i.e. significantly below levels of concern [13].

g. Predictability of effect

The selected LTX-109 concentration and dosing regimen have been determined based on efficacy and safety data from *in vitro* and *in vivo studies* as described earlier, summarized in the Investigators Brochure and the previously cited reports. LTX-109 0.5% formulation has been demonstrated effective in hamsters. For SARS-CoV-2 the $IC_{99.9}$ (inhibitory concentration) is 0.3% and the IC_{50} is 0.1% following 10 minutes exposure. For the tested Influenza A strains and for Influenza B the $IC_{99.9}$ is <0.3%. The chosen dosing regimen is similar to the regimen used in the *in vivo* hamster and ferret studies but with a lower frequency, because of 1) the longer viral replication cycle in humans compared to hamsters, and 2) for compliance reasons [22].

h. Interaction with other products

No interactions known or expected.

i. Managing of effects

In three nasal decolonization studies with multiple intranasal administrations of LTX-109 gel formulation, nasal discomfort and pruritus occurred more frequently in the LTX-109 treatment group; both side-effects were well-tolerated. So, there is a risk of transient discomfort (i.e. itching or burning sensation) during the first few minutes after LTX-109 nasal administration.

Patients will be informed about these possible side-effects in the Patient Information Sheet and verbally. Patients take their first dose during the inclusion with their GP. No effects that need medical interference are expected.

Participants will, however, be instructed to discontinue treatment and contact their GP, in case of excessive and/or persistent nasal pain, or an excessive and/or persistent stinging sensation in their nose.

(Serious) Adverse Events will be reported via the diary, phone calls and upon contact between patient and physician and monitored throughout the trial by the medical monitor and DSMB.

j. Study population

Pregnant/breastfeeding women and WOCBP not adequately protected against pregnancy will be excluded, because not enough safety data is available for this population (see section 13.2).

13.2 Overall synthesis of the direct risks for participants

TableError! Reference source not found. summarises risks that have become apparent in the pre-clinical phase of the LTX-109 development program.

Table 1. Summary of prior and ongoing risks.

Potential Risk	Preclinical data	Clinical data	Actions
Teratogenicity	No data	No data	Adequate contraception and a negative pregnancy test of WOCBP. No breastfeeding females are allowed.

Genetic Toxicology data

Reproductive toxicology data are not yet available for LTX-109. Therefore, women that are currently pregnant or breastfeeding will be excluded, and measures preventing a pregnancy in female participants during trial participation will be implemented at this stage in line with ICH M3 (R2) guidelines.

The potential genotoxicity of LTX-109 has been assessed *in vitro* and *in vivo* by genotoxicity studies. The *in vitro* studies were a Chromosomal Aberration Assay [10] and a Mouse Lymphoma Mutation Study [11]. The Chromosomal Aberration Assay used Chinese hamster ovary cell cultures and showed no evidence of LTX-109 inducing structural chromosomal aberrations, or any increases in polyploidy. It was concluded that LTX-109 was not clastogenic towards Chinese hamster ovary cells. The Mouse Lymphoma Mutation study showed no indications of mutagenic activity by LTX-109. *In vivo* genotoxic potential of LTX-109 was assessed in a Rat Micronucleus Study [12]. The study concluded that LTX-109 does not induce micronuclei in bone marrow cells when tested at the maximum tolerated dose of 7.5 mg/kg in male and female Caesarian Derived Sprague-Dawley rats. Concluding, the *in vitro* and *in vivo* genotoxicity tests do not indicate any genotoxic potential for LTX-109.

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