Study Title	Evaluation of Safety and Pharmacodynamics of OP0201 Compared to Placebo in Healthy Adults
NCT Number	NCT03828149
Document Description	Clinical Protocol, Version: V04_0
Document Date	08 Jan 2019

TRIAL PROTOCOL

EVALUATION OF SAFETY AND PHARMACODYNAMICS OF OP0201 COMPARED TO PLACEBO IN HEALTHY ADULTS

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(Sponsor)
represented by
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[redacted]

Novus TherapeuticsTrial Protocol Code: OP0201-C001

University of Cologne Internal Trial Protocol Code: Uni-Koeln-2809

EudraCT number: 2016-003667-19

08-01-2018, Version V04_0

I. Signatures

[redacted]

Signature Date

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Signature Date

[redacted]

Signature Date

[redacted]

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II. Synopsis

Sponsor:	Novus Therapeutics, Inc. 19900 MacArthur Blvd., Suite 550 Irvine, California 92612, U.S.A. represented in the EU by University of Cologne Albertus-Magnus-Platz 50923 Cologne Germany
[redacted]	[redacted]
Title of the clinical trial:	Evaluation of Safety and Pharmacodynamics of OP0201 Compared to Placebo in Healthy Adults
Indication:	Eustachian tube function

Phase:	I
Type of trial, trial design, methodology:	Single center clinical trial Randomized, double-blind, placebo-controlled, single dose, cross-over
Number of subjects:	16
Primary trial objective:	To evaluate the safety and tolerability of a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.
Secondary trial objectives:	1. To assess pharmacodynamics of the Eustachian Tube following a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults. 2. To explore whether a single intranasal dose of OP0201 compared to a single intranasal dose of placebo modulates ear pain during the hypobaric/hyperbaric atmospheric pressure chamber assessment in healthy adults.
Trial end points:	Variables to be evaluated in support of the primary objective (safety and tolerability) for this study: Adverse events Otoscopy Tympanometry Nasal and Epipharynx Endoscopy Triplicate 12-Lead ECG Physical exam Vital signs Clinical laboratory tests Variables to be evaluated in support of the secondary objectives (pharmacodynamics [continuous tympanic impedance] and ear

pain) for this study:

Pharmacodynamics

- Eustachian tube opening pressure (ETOP)
- Eustachian tube opening duration (ETOD)
- Eustachian tube closing pressure (ETCP)
- Eustachian tube opening frequency (ETOF)

Ear pain

Diagnosis and Principal inclusion and exclusion criteria:

Medical condition or disease to be investigated:

None; this is a healthy volunteer trial.

Key Inclusion criteria:

- Male and female; 18 to 50 years of age at time of signed informed consent
- Healthy subjects with no history or presence of significant medical condition or a clinically significant abnormal finding, as determined by the investigator. This includes study screening results from:
  - Medical history
  - Physical examination
  - ECG (in consultation with cardiologist)
  - Vital sign measurements
  - Clinical laboratory data
- Physiologic tympanogram type A or type C[1] at screening visit

Key Exclusion criteria:

- Upper respiratory tract infection currently or within 6 weeks prior to screening visit

[1] Tympanogram Type C defined as peak pressure between -100 mm water to -200 mm water

- Allergy or sinus conditions (e.g., sinusitis, non-specific nasal inflammation) currently or within 6 weeks prior to screening visit
- Clinically significant findings on ENT exam
- Gastroesophageal reflux disease currently or within 6 weeks prior to screening visit
- Current diagnosis of sleep apnea
- Evidence of craniofacial anomalies (eg, cleft palate, Down's Syndrome) that may interfere with Eustachian Tube function
- Disorders with decreased mucociliary clearance or higher viscosity of the mucous (eg, cystic fibrosis, primary ciliary dyskinesia, Kartagener's syndrome)
- Clinically significant ear disease currently or within 6 weeks prior to screening visit
- History of tympanostomy tubes in one or both ears within 48 weeks prior to screening visit
- Clinically relevant blockage of one or both nasal passages, in the investigator's opinion
- Females who are pregnant, nursing, or planning a pregnancy during the trial

Name of investigational medicinal product (IMP):	OP0201 [dipalmitoylphosphatidylcholine (DPPC):cholesteryl palmitate (CP)]
Investigational medicinal product – dosage and method of administration:	• OP0201 (DPPC:CP [approximately 20:1 w/w] in HFA-134a) metered-dose inhaler • Intranasal administration • Dosage: 20 mg OP0201 administered as four consecutive sprays (2.5 mg per spray) first to the left nare, followed by four consecutive sprays to the right nare until a total of eight sprays (four to each nare) has been administered (see Investigational Medicinal Product (IMP) Instructions for Use).
IMP or therapy used as a comparator – dosage and method of administration:	• OP0201 Placebo (HFA-134a only) metered dose inhaler • Intranasal administration • Dosage: OP0201 Placebo administered as four consecutive sprays (0 mg per spray) first to the left nare, followed by four consecutive sprays to the right nare until a total of eight sprays (four to each nare) has been administered (see Investigational Medicinal Product (IMP) Instructions for Use).
Duration of application:	Trial subjects will receive a single-dose of OP0201 or OP0201 Placebo at the Day 1 visit, followed by an approximately one-week wash-out, and then they will receive a single-dose of the opposite IMP at the Day 8 cross-over visit. It is anticipated that the subject will be in the clinic for approximately 4 to 6 hours for each trial visit.
Estimated Time plan:	First patient first visit (FPFV): Q1 2019 Last patient first visit (LPFV): Q1 2019 Last patient last visit (LPLV): Q1 2019 End of trial: Q1 2019 Final trial report to BfArM: Q1 2020 (See Chap. 3.1.1.)

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[redacted]

Statistical methods: A centralised blocked 2-group randomization stratified by gender and “non-significant baro-challenges (yes/no)“ will be performed via the internet based randomization service TENALEA.

Populations:

The safety population will consist of all subjects who received at least one spray of IMP in either nare. In safety data analyses, subjects will be analysed according to the actual IMP received, regardless of randomization assignment.

The per-protocol population (PP) will consist of all randomized subjects who received the two complete doses (4 sprays to each nare) of IMP at Day 1 and 8. Valid measures of ETOP and ETOD for passive phases 1 and 5 for both ears and for all three measurement times at visits 2 and 4 Excluded are subjects with major protocol deviations (determined prior to database lock and unblinding of the data).

Analyses:

The analysis will be descriptive, significance level will not be adjusted for multiple testing. A p-value ≤ 5% is defined as significant for demographic and screening characteristics and the assessments supporting the secondary objectives for this study.

Descriptive summary tables for each treatment and period and for each measurement time will be performed including statistics depending on variable type. If variables are measured before and after treatment at the same visit, the difference will be calculated for each visit and analyses will apply to this change, if appropriate.

Incidence of AEs will be tabulated by primary system organ class (SOC) and by preferred term within each primary SOC.

Primary analysis is the evaluation of the safety variables in the safety population. Adequate confidence intervals will be given for the change from baseline, if appropriate.

Secondary analysis consisting of pharmacodynamics and ear pain analyses will be performed in the safety population and additionally in the PP population. Non-parametric treatment comparisons will be done with a Grizzle's two-stage approach for the cross-over design. The tests will be carried out without covariates. If confidence intervals (CI) are to be provided for 2-group comparison, the 95% 2-sided Hodges-Lehmann intervals will be calculated.

Missing values will not be replaced, but drop-outs may be replaced with additionally randomized trial subjects to ensure N=16 for the PP population.

Subgroup analyses are planned for stratification factors, if sufficient numbers of subjects are met.

Sample size:

N=16 in the PP population.

GCP conformance: This protocol is to be conducted in accordance with the applicable current Good Clinical Practice (GCP) regulations and guidelines, i.e., the International Council for Harmonization (ICH) Guideline on GCP.

Financing: Novus Therapeutics, Inc.

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IV. Abbreviations

Abbreviation	meaning
ADR	Adverse Drug Reaction
AOM	Acute otitis media
AE	Adverse Event
AMG	*Federal Drug Law* (Arzneimittelgesetzt)
BID	Twice daily
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
CA	Competent authority (BfArM)
BP	Blood pressure
CI	Confidence interval
CP	Cholesteryl Palmitate
CRF	Case Report Form
CTCC	Clinical Trial Centre Cologne
DMC	Data Monitoring Committee
DPPC	Dipalmitoylphosphatidylcholine
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
ENT	Ear, nose & throat

EOS	End-of-trial
ET	Eustachian tube
ETCP	Eustachian tube closing pressure
ETOD	Eustachian tube opening duration
ETOF	Eustachian tube opening frequency
ETOP	Eustachian tube opening pressure
ETD	Eustachian tube dysfunction
FPFV	First patient first visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HFA-134a	Hydrofluoroalkane 134a (1,1,1,2-tetrafluorethane)
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IMSB	Institute for Medical Statistics and Computational Biology
ITT	Intent-to-treat
ISF	Investigator Site File
LKP	Leiter der klinischen Prüfung (Principal Coordinating Investigator)
LPFV	Last patient first visit
LPLV	Last patient last visit
ME	Middle ear
mg	Milligram(s)

mL	Milliliters
mmHG	Millimeters of mercury
OM	Otitis media
OME	Otitis media with effusion
OTC	Over-the-counter
PCI	Principal Coordinating Investigator (Leiter der klinischen Prüfung)
PE	Physical Exam
PL	phospholipid
PP	Per-protocol
PR	Pulse rate
QD	Once daily
RR	Respiratory rate
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SDV	Source data verification
SOC	System organ class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TM	Tympanic membrane
TMF	Trial Master File
T	Temperature
w/w	Weight/weight

1 Introduction

1.1 OP0201 (dipalmitoylphosphatidylcholine [DPPC] and cholesteryl palmitate [CP])

The Investigational Medicinal Product (IMP), OP0201, is comprised of two active ingredients, dipalmitoylphosphatidylcholine (DPPC [a phospholipid surfactant; also known as colfosceril palmitate]) and cholesteryl palmitate (CP [a neutral phospholipid spreading agent]), in approximately 20:1 (weight/weight [w/w]) ratio, and an inactive ingredient, synthetic chlorofluorocarbon-free propellant, hydrofluoroalkane 134a (HFA-134a). The product is delivered to the nasal mucosa in the anterior nares, towards the opening of the Eustachian tube (ET), as a dry powder in HFA-134a via an intranasal metered dose inhalant (MDI). Each 0.1 milliliter (mL) MDI spray of OP0201 delivers 0.1 mL of HFA-134a containing approximately 2.5 milligrams (mg) of the active ingredients (approximately 2.38 mg of DPPC and approximately 0.12 mg of CP).

For further details about OP0201 see Investigator's Brochure.

1.2 Rationale for the clinical trial

The ET is a compliant liquid-lined tube between the middle ear (ME) and the nasopharynx. The ET serves three major physiologic functions: 1) ventilation of the ME to maintain ambient air pressure, 2) clearance of fluid from the ME to nasopharynx, and 3) protection of the ME (1, 2). It is well established that Eustachian Tube Dysfunction (ETD) is a significant factor in the cause of middle ear diseases, such as acute and chronic otitis media (1, 3, 4).

During the past few decades, various methods have been developed to evaluate ET function such as manometric, endoscopic and sonographic methods, impedance measurements and Tubomanometry. However, none of these methods was able to reflect physiologic conditions and fully assess ET function (5, 6). Another established method for evaluating ET function is by utilizing a pressure chamber (7, 8).

It is established that surfactants, which are endogenous components in human ETs, are present on the liquid surface of the ET's mucosal lining. Surfactants lower surface tension. Thus, when surfactant is administered exogenously to ET, it is hypothesized that it will spread along the pressure gradient from the area of high surface pressure (low surface tension) to an area of low surface pressure (high surface tension) and thus may act as 'release' agents by being directly absorbed onto the ET surface, causing the entire surface to become hydrophobic. As a result, ET function is modulated. It has been demonstrated in various animal models, including healthy animals, that exogenous surfactant administered through the nose to the ET reduces the ET

surface tension, which in turn decreases the ET passive opening pressure, facilitating opening of the ET (2).

This exploratory trial is proposed as an evaluation of the safety and tolerability of intranasal administration of OP0201 in healthy adults. In addition, the trial will evaluate pharmacodynamics of the ET to guide future studies of OP0201 as a possible treatment for diseases caused by ET dysfunction (e.g. Otitis Media).

1.3 Primary objective

The primary objective of the trial is to assess the safety and tolerability of a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.

1.4 Secondary and other objectives

The secondary objectives in this trial are:

1. To assess pharmacodynamics of the ET following a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.

2. To explore whether a single intranasal dose of OP0201 compared to a single intranasal dose of placebo modulates ear pain during the hypobaric/hyperbaric atmospheric pressure chamber assessment in healthy adults.

1.5 Benefit-Risk Analysis

This trial is designed to evaluate the safety, tolerability, pharmacodynamics of the ET and potential effect in modulating ear pain of an investigational treatment (OP0201) in healthy volunteers. A benefit-risk analysis is not able to be fully established at this time. There is no expected or anticipated benefit to healthy subjects. The IMP is not expected to cause harm to healthy subjects. Both DPPC and CP are endogenous to human nasal passages and ETs. Furthermore, surfactants in much higher concentrations and doses are components in several pharmaceutical products that have received approval by Health Authorities, including US FDA and many EU countries, (e.g., Alveofact®[2], Curosurf®, Infasurf®, Survanta®) as intratracheal treatment of premature infants with respiratory distress syndrome (RDS). The propellant excipient, HFA-134a, is used in approved metered dose oral inhalers (e.g., Proventil HFA® [albuterol], Aerospan® [flunisolide] and intranasal inhalers (e.g., QNASL® [beclomethasone]) containing active pharmaceutical agents for the

[2] Alveofact® and Curosurf® are both approved and marketed in Germany

management of asthma and chronic obstructive pulmonary disease (see Investigator's Brochure, Table 5, Section 8.1)). The MDI device used to deliver OP0201 into the nares is similar to devices utilized in the delivery of other approved inhaled metered dose products.

2 Organisational and administrative aspects of the trial

2.1 Sponsor

Sponsor: Novus Therapeutics, Inc.
19900 MacArthur Blvd., Ste. 550
Irvine, CA 92612
United States

Represented by: University of Cologne
Albertus-Magnus-Platz
50923 Cologne
Germany

2.2 Principal Coordinating Investigator

[redacted]

2.3 Statistics

[redacted]

2.4 Data Monitoring Committee

A Data Monitoring Committee (DMC) is not planned for this trial.

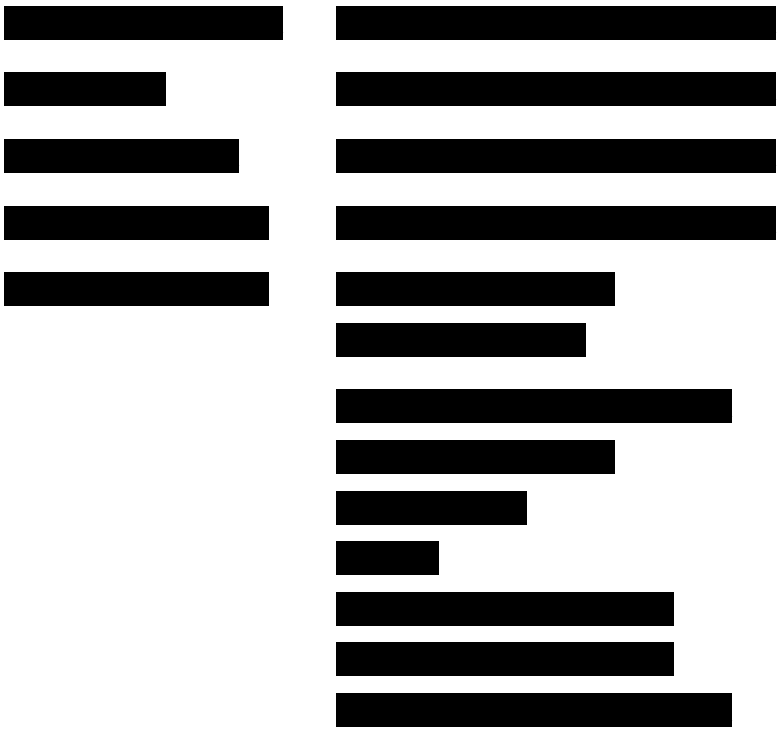
2.5 Trial laboratories and other technical services

The local laboratory will perform the laboratory analyses described in this protocol.

The local pharmacy will be responsible for storing and dispensing the OP0201 IMP during the trial. They will also be responsible for maintaining a detailed inventory of the OP0201 IMP including accounting for the number of OP0201 IMP units received, the number of OP0201 IMP units dispensed, and the number of unused OP0201 IMP units at the completion of the trial.

An ECG vendor, ERT (Peterborough, United Kingdom) will be responsible for ECG data review and analysis.

2.6 Central organisation units



2.7 Investigators and trial sites

This clinical trial will be conducted at a single centre

A listing of the trial site, (principal) investigators, their deputies, subinvestigators, and other trial staff, will be kept and continuously updated. The final version of this list will be attached to the final report of the clinical trial.

2.7.1 Requirements for investigators and trial sites

The clinical trial site where the trial will be conducted has to meet the following requirements as agreed by the Sponsor and University of Cologne:

- Specialised unit for ear, nose and throat discipline
- Possession of a CE-certified single-person hypobaric and hyperbaric pressure chamber capable of performing compression and decompression phases between 0.8 and 1.2 bar relative to atmospheric pressure in order to assess continuous tympanic impedance;
- Space and facilities to perform standard ENT exams including tympanogram, ear microscopy and nose endoscopy.
- Facilities to conduct monitoring of the clinical trial.

The investigators have to meet the following requirements for the conduct of the trial as agreed by the Sponsor and University of Cologne:

- Specialist ENT physician with at least 3 years professional experience (principal coordinating investigator, PCI)
- At least 2 years experience in the conduct of clinical studies according to GCP as member of investigator's team or investigator (PCI)
- Registered physician with at least 1 year professional ENT experience (subinvestigator)
- Experienced in using the hypobaric/hyperbaric pressure chamber
- Trained and/or experience in regulatory requirements of clinical trials according to the German Federal Medicines Law ("Arzneimittelgesetz") and GCP

In addition, requirements for investigator and subinvestigator will be described in detail on a separate qualification list.

2.8 Financing

The clinical trial is funded by Novus Therapeutics, Inc. Additionally, the IMP is provided free of charge by Novus Therapeutics, Inc.

3 Trial conduct

3.1 General aspects of trial design

This is a randomized, double-blind, placebo-controlled, cross-over trial to evaluate the safety, tolerability, pharmacodynamics of the ET and potential effect in modulating ear pain of a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.

3.1.1 Estimated Time plan

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

End of the clinical trial

The end of trial is defined as the last patient last visit (LPLV).

3.2 Discussion of trial design

This is a randomized, double-blind, placebo-controlled, cross-over trial designed to evaluate the safety, tolerability, pharmacodynamics of the ET and potential effect in modulating ear pain of a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.

A total of 16 subjects will be randomized to a treatment sequence – either OP0201-placebo or placebo-OP0201. Each subject will participate in the trial for up to five weeks and have up to three in-clinic visits and two telephone follow-up visits. There will be a screening period of up to 28 days, with a randomization visit on Day 1 where subjects will be randomized to receive a single masked dose of intranasal OP0201 containing a total of 20 mg OP0201 or placebo. The trial site staff will administer the IMP to each subject as four consecutive sprays at a time into each nare so that a total of eight sprays (four per nare) have been administered. On Day 8, subjects will return to the trial site for the cross-over visit where they will receive the opposite masked treatment

administered in the same way. Subjects will remain in the clinic for the duration of approximately 4 to 6 hours at each visit.

Safety will be assessed by clinical monitoring and will include a general physical examination, an ear, nose and throat (ENT) examination that includes otoscopy, nose and epipharynx endoscopy, tympanogram, clinical laboratory tests (hematology, biochemistry and urinalysis), triplicate12-lead ECG, vital signs (PR, RR, BP, temperature), and spontaneous adverse event reporting.

Pharmacodynamics of OP0201 on ET function will be assessed by continuous tympanic impedance measures using a single-person hypobaric/hyperbaric atmospheric pressure chamber.

An exploratory efficacy assessment of OP0201 effect to modulate ear pain during the hypobaric/hyperbaric atmospheric pressure chamber protocol is also planned.

3.3 Selection of trial population

Prospective subjects who meet inclusion and who do not meet exclusion criteria as defined in Sections 3.3.1 and 3.3.2 (inclusion/exclusion, respectively) will be considered for entry into this trial.

Subject eligibility should be reviewed and documented by an appropriately qualified member of the investigator's trial team before subjects are included in the trial. The investigator or other medically qualified individual is responsible for assuring that subjects meet eligibility criteria.

3.3.1 Inclusion criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrolment into the trial:

- Male and female; 18 to 50 years of age at time of signed informed consent
- Written informed consent obtained
- Body Mass Index (BMI) 18 to 30 and a minimum body weight of 50 kg at the screening visit
- Healthy subjects with no history or presence of significant medical condition or a clinically significant abnormal finding, as determined by the investigator. This includes study screening results from:
    - Medical history
    - Physical examination
    - ECG (in consultation with cardiologist)
    - Vital sign measurements

- Clinical laboratory data

- Negative urine pregnancy test at screening and baseline for females of childbearing potential
- Agree to refrain from water immersion of the ears from the time of signed informed consent to the end of trial
- Physiologic tympanogram type A or type C[3] at screening visit
- Ability to follow trial instructions and complete all required visits

3.3.2 Exclusion criteria

If any of the following criteria are met, the subject is not eligible for participation in the trial.

- Known substance abuse (e.g., alcohol, licit or illicit drugs) within 96 weeks prior to screening visit
- Positive urine drug screen at screening visit
- Upper respiratory tract infection currently or within 6 weeks prior to screening visit
- Allergy or sinus conditions (e.g., sinusitis, non-specific nasal inflammation) currently or within 6 weeks prior to screening visit
- Claustrophobia that is sufficient to prevent them tolerating assessments while in a hypobaric/hyperbaric atmospheric pressure chamber
- Smoker (e.g., cigarettes, vapor) within the last 48 weeks prior to screening visit
- Clinically significant findings on ENT exam
- Gastroesophageal reflux disease currently or within 6 weeks prior to screening visit
- Current diagnosis of sleep apnea
- Evidence of craniofacial anomalies (eg, cleft palate, Down's Syndrome) that may interfere with ET function
- Disorders with decreased mucociliary clearance or higher viscosity of the mucous (eg, cystic fibrosis, primary ciliary dyskinesia, Kartagener's syndrome)
- Known hypersensitivity to any of the ingredients in the investigational product
- Clinically significant ear disease currently or within 6 weeks prior to screening visit

[3] Tympanogram Type C defined as peak pressure between -100 mm water to -200 mm water

- History of tympanostomy tubes in one or both ears within 48 weeks prior to screening visit
- Clinically relevant blockage of one or both nasal passages, in the investigator's opinion
- Use of any medication (either topically or systematically) for a condition related to ear or nose currently or within 12 weeks prior to screening visit
- Use of anti-cholinergic medication (eg, anti-histamine, anti-nausea) currently or within 6 weeks prior to screening visit
- Within seven days prior to the first study drug administration, use of any prescription drugs (except for oral contraceptives), and/or any over-the-counter drugs including herbal supplements, vitamins, dietary supplements (except for the occasional use of paracetamol not exceeding 3 grams per day)
- Any condition or situation that would, in the investigator's opinion, put the subject at significant risk, confound the trial results or interfere significantly with the subject's participation
- Participation in a clinical trial within 30 days prior to screening visit
- Females who are pregnant, nursing, or planning a pregnancy during the trial
- Females of child-bearing potential who are not willing to use an acceptable method of contraception during the trial (see Section 3.6.9 Pregnancy and Contraception Considerations).
- Investigator site personnel directly affiliated with this trial and/or their immediate families (defined as spouse, parent, child, or sibling, whether adopted or biologic).
- Persons employed by the Sponsor or investigator
- Persons held in an institution by legal or official order

3.4 Withdrawal of trial subjects after trial start

The subject's participation in the trial is voluntary. The subject may refuse to participate or withdraw from the trial at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects may be withdrawn at the discretion of the Investigator or Sponsor for safety, behavioural reasons, or the inability of the subject to comply with the protocol required schedule of trial visits or procedures at the trial site.

Notification of early subject withdrawal from the trial and the reason for discontinuation will be clearly documented in the electronic case report form (eCRF).

Subjects who withdraw from the trial may be replaced at the discretion of the investigator upon consultation with the Sponsor.

3.4.1 Procedures for premature withdrawal from treatment during the trial

If a subject does not return for a scheduled visit (crossover), every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal, request the subject to participate in the final visit, if applicable, and follow-up with the subject regarding any unresolved adverse event, if any occurred.

If the subject withdraws from the trial and also withdraws consent for disclosure of future information, no further evaluations should be performed, no additional data should be collected. If such action is taken, the date of withdrawal of consent must be documented. The Sponsor may retain and continue to use any data collected prior to such withdrawal of consent.

All data collected until this point of time will be stored according to AMG §40, 2a, 3. If a subject is lost to follow up, i.e. not attending the visit on day 8 (crossover), then the subject will be withdrawn by the investigator, and replaced.

3.5 Closure of trial sites/Premature termination of the clinical trial

3.5.1 Closure of trial sites

The trial site will be closed in case of insufficient subject recruitment in a timely manner, or by mutual decision of the investigator/PCI and Sponsor for any reason.

3.5.2 Premature termination of trial

The Sponsor has the right to terminate the trial prematurely if there are any relevant medical or ethical concerns, or if completing the trial is no longer practical. If such action is taken, the reasons for terminating the trial must be documented. All trial subjects still under treatment at the time of termination should undergo a final examination, which should be documented.

3.5.3 Stopping Criteria for individual subjects and for the study

The following stopping criteria are defined:

1. Stopping Criteria for Individual Subjects

   Study treatment for any individual subject will be stopped if the subject experiences a serious adverse event (SAE) or a clinically significant possibly drug-related related AE, which in the

opinion of the study physician, Principal Investigator, or Sponsor's medical representative, warrants discontinuation of the study for that subject's wellbeing.

Any female subject who becomes pregnant whilst participating in the study will be withdrawn from the study.

2. Criteria for Stopping the Study

If 1 or more subjects experience drug-related SAEs or 2 or more subjects experience any drug related severe AEs, the study will be stopped.

If any of the following scenarios occur with a reasonable possibility of a causal relationship with the study treatment, the study will be stopped:

- ≥1 subject experiences SAEs considered to have a reasonable possibility of relationship to the study treatment.
- ≥2 subjects experience non-tolerable AEs that are considered to have a reasonable possibility of relationship to the study treatment.
- ≥1 subject receiving study treatment fulfills Hy's Law defined as alanine aminotransferase (ALT) >3 × the upper limit of normal (ULN) and bilirubin >2 × ULN in the absence of significant increase in alkaline phosphatase (ALP) and in the absence of an alternative diagnosis that explains the increase in total bilirubin, to be assessed from the first administration of study treatment up to and including follow-up.
- ≥2 subjects who receive study treatment have >2 ULN of either ALT, bilirubin, or ALP.
- ≥2 subjects who receive study treatment have a QTc prolongation defined as QTcF >500 ms, or an increase of QTcF >60 ms above baseline on the 12-lead electrocardiogram (ECG), confirmed (persistent for >5 minutes) on repeated 12-lead ECGs.

3.6 Treatment

3.6.1 Treatments to be given

The trial site staff will administer each dose to the subject per the detailed instructions for IMP administration provided in the IMP Instructions for Use.

At Day 1, subjects will receive a single masked dose of either OP0201 20 mg (2.5 mg per spray) or placebo (0 mg per spray) administered as four consecutive sprays first to the left nare, followed by four consecutive sprays to the right nare until a total of eight sprays (i.e. four to each nare) has been administered. At Day 8, subjects will return to the trial site where they will receive the opposite masked treatment administered as four consecutive sprays first to the left nare, followed by four consecutive sprays to the right nare until a total of eight sprays (i.e. four to each nare) has been administered.

Figure 1: Trial flowchart

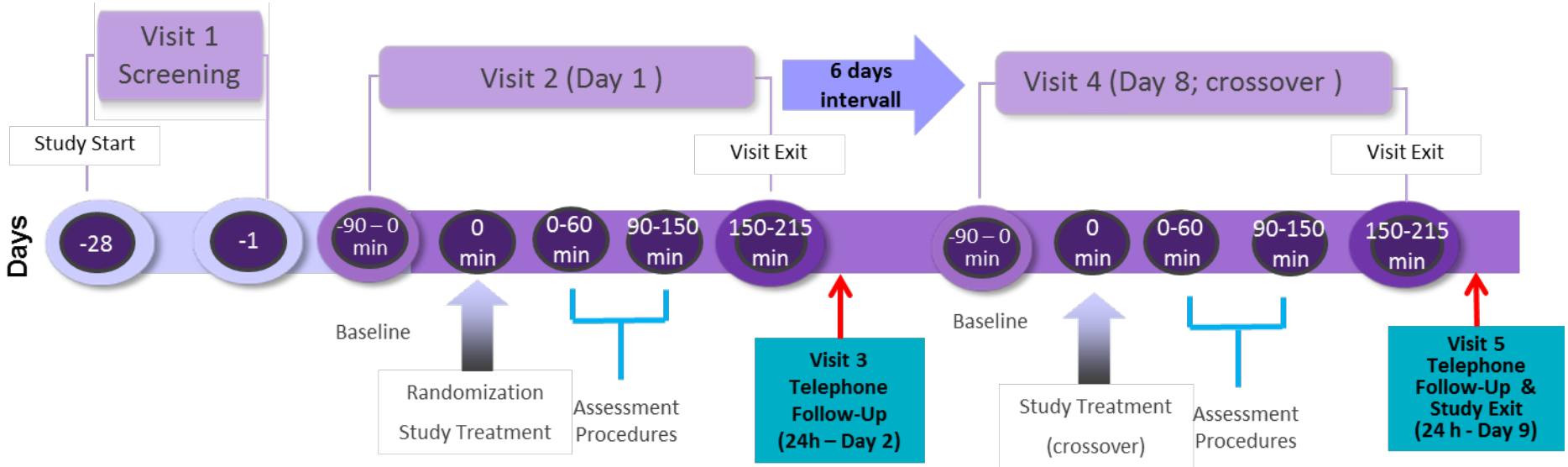


Table 1: Visit Schedule

Assessment Procedures	Screening (-28 days to -1 day)	Day 1 (Baseline) -90-0 min	Day 1 (Trial Treatment) 0 min	Day 1			Day 2[h]	6 days interval	Day 8 ± 1 (Baseline; crossover) -90-0 min	Day 8 ± 1 (Trial Treatment; crossover) 0 min	Day 8 ± 1 (crossover)			Day 9[h] (Trial Exit)
				0-60 min	90-150 min	150-215 min (Visit Exit)					0-60 min	90-150 min	150-215 min (Visit Exit)	
Informed Consent	X													
Demographics	X													
Inclusion/Exclusion	X	X												
Medical, Surgical and Ear History[i]	X													
Physical Exam[a]	X												X	
Tympanogram	X	X				X			X				X	
Continuous measure of tympanic impedance (ETOP, ETOD, ETCP, ETOF) in hyperbaric/hypobaric pressure chamber	X	X		10 Min ± 5[g]	X				X		10 Min ± 5[g]	X		
Ear pain assessment (Numeric Pain Rating Scale 0-10)		X		X	X				X		X	X		
Otoscopy incl. TEED classification[b]	X	X		X	X				X		X	X		
Nose and epipharynx endoscopy[j]	X	X			X				X			X		
12-Lead ECG	X	X[k]				X[k]			X[k]				X[k]	
Clinical laboratory tests[c]	X												X	
Vital signs[d]	X	X				X			X				X	
Urine sample for drug screen	X													
Urine Pregnancy tests[e]	X	X							X					
Concomitant medications/procedures	X	X				X	X		X				X	X
Randomization		X												
IMP Administration[f]			X							X				
Adverse Events	X	X	X	X	X	X	X		X	X	X	X	X	X

ETOP = Eustachian Tube Opening Pressure; ETOD = Eustachian Tube Opening Duration; ETCP = Eustachian Tube Closing Pressure; ETOF = Eustachian Tube Opening Frequency; ECG = Electrocardiogram; IMP = Investigational Medicinal Product.

a. Includes: general appearance, overall status of the skin, head, neck, trunk, eyes, heart and lungs (eg, breathing sounds), abdomen, extremities, lymph nodes. Height and weight are recorded only on day of screening visit.
b. Otoscopy involves ear endoscopy, taking a picture of the tympanic membrane and assessing TEED classification (TEED 0-4).
c. Includes hematology, biochemistry and urinalysis
d. Includes blood pressure (BP) [mmHg], respiration rate (RR) [breaths/min], pulse rate (PR) [beats/min] and body temperature (T) [°C].
e. For females of childbearing potential; urine pregnancy test must be negative.
f. Start time of IMP administration and stop time of last spray must be recorded.
g. Tympanic impedance measures are taken 10 ± 5 min after administration of IMP is completed. Time points as follows are recorded: (1) start time of impedance measures and (2) stop time of impedance measures.
h. Telephone follow-up asking subjects about general status of well-being 24 h after IMP administration
i. Ear history will record subject's response (yes/no) to the following question "Have you ever had problems (eg, symptoms of aural fullness, popping or discomfort/pain) when flying or diving?").
j. Nose endoscopy will include a measurement of nasal cavity length (in cm) only on day of screening visit.
k. Triplicate 12-Lead ECG

3.6.2 Visit Schedule

Unless otherwise defined as in Figure 1 Flow Chart and Table 2 Visit Schedule, the following list of study-related procedures do not reflect a particular order. The specific order of sequence for these study-related procedures are referenced in an operational study procedure manual.

3.6.2.1 Screening

Subjects will be screened within 28 days up to 1 day prior to randomization to confirm that they meet the subject selection criteria for the trial. Prior to performing any study related procedure, the investigator (or an appropriate delegate at the trial site) will obtain informed consent from each subject according to the process as described in Section 5.4.

The following procedures will be completed during the screening period:

- Obtain written informed consent
- Record demographics (age, race, gender)
- Record height in centimeters (cm) and weight in kilograms (kg)
- Assess inclusion/exclusion criteria
- Record medical, surgical and ear history
- Record subject's response (yes/no) to the following question "Have you ever had problems (eg, symptoms of aural fullness, popping or discomfort/pain) when flying or diving?")
- Perform physical examination (general appearance, overall status of the skin, head, neck, trunk, eyes, heart and lungs (eg, breathing sounds), abdomen, extremities, lymph nodes)
- Obtain vital signs (PR [beats/min], RR [breaths/min], BP [mmHg], T [°C]))
- Perform otoscopy (separately for left and right ear, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1)
- Perform nasal endoscopy (separately for left and right nostril; assess as normal/abnormal)
- Perform epipharynx endoscopy (assess as normal/abnormal)
- Record measurement of nasal cavity length [cm] (separately for left and right side)
- Perform tympanogram (assess as Type A, Type B or Type C)
- Perform tympanic impedance measurements in hypobaric/hyperbaric pressure chamber (see Attachment 9.1)
- Collect 12-Lead ECG
- Collect clinical laboratory tests (non-fasting), (assess as normal/abnormal – if abnormal, determine if clinically significant [yes/no], parameters described in Section 3.7.1)
- Collect urine for drug screen

- Perform urinary pregnancy test for females of child-bearing potential performed, record as negative/positive
- Record adverse events, if applicable
- Record previous medication used within 12 weeks prior to screening and concomitant medications/procedures, if applicable
- Confirm subjects agree that they will need to avoid immersion of ears in water for the course of the study.

At the end of the screening visit, potential subjects will be instructed to;

- Avoid immersion of ears in water between now and the time they return for the Day 1 visit until completion of the in-clinic visit on Day 8
- Refrain from the following within 24 hours prior to Day 1 and again 24 hours prior to Day 8:
    - Consumption of more than six units (cups/glass) per day of coffee, tea, cola, energy-drinks, or other caffeinated beverages or foods containing caffeine or xanthine
    - Consumption of alcohol
    - Unaccustomed strenuous physical activity (e.g., weight lifting, running, bicycling, etc.).
- Avoid the following medications throughout the duration of the trial:
    - Medications (either topically or systematically) for a condition related to ear or nose.
    - Anti-cholinergic medications (eg, anti-histamine, anti-nausea)
    - Any prescription drug (except oral contraceptives, see Section 3.6.9)
    - Any over-the-counter (OTC) drugs including herbal supplements, vitamins, dietary supplements (except for the occasional use of paracetamol not exceeding 3 grams per day)

3.6.2.2 Day 1

During the time subjects are in the clinic on Day 1, food or drink should not be consumed, other than water.

Day 1 Baseline (-90 to 0 min pre-dose)

- Confirm inclusion/exclusion criteria
- Perform urinary pregnancy test for females of child-bearing potential performed, record as negative/positive
- Obtain vital signs (PR, RR, BP, T)
- Collect triplicate 12-Lead ECG

- Perform otoscopy separately for left and right ear, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1
- Perform nasal endoscopy (separately for left and right nostril; assess as normal/abnormal)
- Perform epipharynx endoscopy (assess as normal/abnormal)
- Perform tympanogram (assess as Type A, Type B or Type C)
- Perform tympanic impedance measurements in hypobaric/hyperbaric pressure chamber (see Attachment 9.1)
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Obtain subject randomization
- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

Day 1 Dosing (0 min)

- Trial site staff will administer IMP to subject
- Record start time of IMP administration and stop time of last spray
- Record adverse events, if applicable

Day 1 Post-Dose Period 0-60 min

- Perform the tympanic impedance measurements in hypobaric/hyperbaric pressure chamber within 10 minutes ±5 minutes of dosing (see Attachment 9.1); record start and stop times of impedance measures
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Perform otoscopy separately for left and right ear within 30 min after completion of the pressure chamber protocol, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1
- Record adverse events, if applicable

Day 1 Post-Dose Period 90-150 min

- Perform the tympanic impedance measurements in hypobaric/hyperbaric pressure chamber (see Attachment 9.1)
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Perform otoscopy separately for left and right ear within 30 min after completion of the pressure chamber protocol, classify each ear according to TEED classification [Section

3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1

- Perform nasal endoscopy (separately for left and right nostril; assess as normal/abnormal)
- Perform epipharynx endoscopy (assess as normal/abnormal)
- Record adverse events, if applicable

Day 1 Exit (150-215 min)

- Perform tympanogram (assess as Type A, Type B or Type C)
- Obtain vital signs PR [beats/min], RR [breaths/min], BP [mmHg], T [°C]
- Collect triplicate 12-Lead ECG
- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

3.6.2.3 Day 2 (telephone follow-up visit)

- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

3.6.2.4 Day 8 ± 1 (crossover visit)

During the time subjects are in the clinic on Day 8, food or drink should not be consumed, other than water.

Day 8 Baseline (-90 to 0 min pre-dose)

- Perform urinary pregnancy test for females of child-bearing potential performed, record as negative/positive
- Obtain vital signs PR [beats/min], RR [breaths/min, BP [mmHg], T [°C]
- Collect triplicate 12-Lead ECG
- Perform otoscopy separately for left and right ear, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1
- Perform nasal endoscopy (separately for left and right nostril; assess as normal/abnormal)
- Perform epipharynx endoscopy (assess as normal/abnormal)
- Perform tympanogram (assess as Type A, Type B or Type C)
- Perform tympanic impedance measurements in hypobaric/hyperbaric pressure chamber (see Attachment 9.1)
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

Day 8 Dosing (0 min)

- Trial site staff will administer IMP to subject
- Record start time of IMP administration and stop time of last spray
- Record adverse events, if applicable

Day 8 Post-Dose Period 0-60 min

- Perform the tympanic impedance measurements in hypobaric/hyperbaric pressure chamber within 10 minutes ±5 minutes of dosing (see Attachment 9.1); record start and stop times of impedance measures
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Perform otoscopy separately for left and right ear within 30 min after completion of the pressure chamber protocol, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1
- Record adverse events, if applicable

Day 8 Post-Dose Period 90-150 min

- Perform the tympanic impedance measurements in hypobaric/hyperbaric pressure chamber (see Attachment 9.1)
- Have subject complete ear pain assessment (0 = no pain up to 10 = worst pain imaginable) during pressure chamber protocol
- Perform otoscopy separately for left and right ear within 30 min after completion of the pressure chamber protocol, classify each ear according to TEED classification [Section 3.7.1] and record description of appearance of TM for each ear as described in Section 3.7.1
- Perform nasal endoscopy (separately for left and right nostril; assess as normal/abnormal)
- Perform epipharynx endoscopy (assess as normal/abnormal)
- Record adverse events, if applicable

Day 8 Exit (150-215 min)

- Perform tympanogram (assess as Type A, Type B or Type C)
- Obtain vital signs PR [beats/min], RR [breaths/min], BP [mmHg], T [°C]
- Collect triplicate 12-Lead ECG
- Perform physical examination (general appearance, overall status of the skin, head, neck, trunk, eyes, heart and lungs (eg, breathing sounds), abdomen, extremities, lymph nodes)

- Collect clinical laboratory tests (non-fasting), (assess as normal/abnormal – if abnormal, determine if clinically significant [yes/no], parameters described in Section 3.7.1)
- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

3.6.2.5 Day 9 Trial Exit (telephone follow-up visit)

- Record adverse events, if applicable
- Record concomitant medications/procedures, if applicable

3.6.3 Description of investigational medicinal product (IMP)

The IMP OP0201 is an intranasal aerosol, a dosage form recognized by the US Pharmacopeia, that contains two active ingredients, DPPC and CP, as a suspension in synthetic HFA-134a (1,1,1,2-tetrafluorothane [Hall 2015]), which is utilized as the propellant for the intranasal administration. OP0201 is supplied in mechanical packaging parts that includes a pressurized canister as the primary container, a metering valve that has a crimping seal for secure attachment to the canister, a tip to deliver the drug to the nares and a holder for the canister.

OP0201 is manufactured using Good Manufacturing Processes with development phase appropriate compliance with global Health Authority guidelines (e.g., European Commission Volume 4, Annex 13; U.S. FDA Guidance for Industry: CGMP for Phase 1 Investigational Drugs, July 2008; U.S. FDA Guidance for Industry: Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products, July 2002). The OP0201 canisters are filled to deliver 100 actuations (sprays) per canister. Each metered spray of OP0201 delivers 0.1 mL of HFA-134a containing approximately 2.5 mg of the active ingredients.

All the ingredients in this drug product are synthetic and do not contain any animal or human derivatives.

For further details about OP0201 see Investigator's Brochure.

3.6.3.1 OP0201

Trade name: N/A

INN (International Nonproprietary Name)/active substance:

Dipalmitoylphosphatidylcholine (DPPC) – $C_{40}H_{80}NO_8P$ (MW: 734.039 g/mol)

Cholesteryl palmitate (5-cholestene 3-palmitate; CP) – $C_{43}H_{76}O_2$ (MW: 625.07 g/mol)

ATC-Code: N/A

Presentation: Dry powder suspension in metered dose inhaler

Dose: 20 mg

Manufacturer: Sciarra Laboratories, Inc., Hicksville, New Jersey, United States.

3.6.3.2 Placebo

Trade name: HFA-134a

INN (International Nonproprietary Name)/active substance:

1,1,1,2-Tetrafluoroethane (CH_2FCF_3)

ATC-Code: N/A

Presentation: Propellant in metered dose inhalor

Dose: N/A

[redacted]

3.6.3.3 Manufacture of the IMP

OP0201 is manufactured using Good Manufacturing Processes with development phase appropriate compliance with global Health Authority guidelines (e.g., European Commission Volume 4, Annex 13; U.S. FDA Guidance for Industry: CGMP for Phase 1 Investigational Drugs, July 2008; U.S. FDA Guidance for Industry: Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products, July 2002) by [redacted] in compliance with EU Directives 2001/20/EC and Annex 13 to the European GMPs and the European Clinical Trial Authorization dossier.

See Investigator's Brochure for further information.

3.6.3.4 Labelling of IMP

The IMP will be packaged and labeled in identical appearing canisters. The IMP (OP0201 and Placebo) will be identified as an IMP. The trial number, subject number and kit number will be identified on the unit label.

The IMP will be labeled in accordance with Section 5 of the GCP regulations (GCP-V).

3.6.3.5 Storage of IMP

The IMP must be stored in a secured area at controlled room temperatures between 15 °C and 25 °C (59 °F and 77 °F); excursions are permitted up to 30 °C (86 °F).

3.6.4 Compliance with treatment & dispensing and return of IMP

A detailed inventory of all of the IMP units for this trial must be completed. The investigator must keep an accurate accounting of the number of IMP units for each IMP that are:

- received from Novus Therapeutics (or Novus Therapeutics designee),
- dispensed/administered to the trial subjects,
- Any used IMP at the trial site will be destroyed according to regular disposal measures and local regulations, with the exception that any problem canister (eg, product does not spray out with pushing the valve, or clumps of product versus a 'spray' are noticed) may be returned to the Sponsor.
- Any unused IMP at the trial site may be returned to the Sponsor or designee.

3.6.5 Assignment of trial subjects to treatment groups

Subjects are assigned to one of two treatment sequences (OP0201-placebo or placebo-OP0201) by means of the central 24/7 Internet randomization service TENALEA (FormsVisions BV, Abcoude, NL) on Day 1. TENALEA provides IMP kit IDs for the randomized subjects. Randomization will be blocked and stratified by gender and "non-significant baro-challenges (yes/no)". Additional details will be documented in the randomization manual.

3.6.6 Selection of dosage of investigational medicinal product

In this controlled clinical trial, the proposed dose is 20 mg OP0201 . The clinical safety and tolerability of OP0201 for human use is guided by data from animal studies . These pre-clinical proof-of-concept and safety evaluations were performed in three animal species (i.e., gerbils, mice, and chinchillas) to assess the effects of the GLP material on ET passive opening pressure (POP), OM with effusion (OME), and acute OM (AOM). As compared to no treatment or propellant alone, the administration of the GLP surfactant material produced significant reductions in ET POP as compared to no treatment or propellant alone within 10 minutes of administration of a single 20 mg intranasal aerosolized dose (9) in healthy animals. In the ears of animals with OME, significant reductions in the duration and severity of middle ear effusion were observed with an average of 10.57 days with 10 mg per day (QD group; (approximate average total exposure of 106 mg) and average of 8.57 days with 20 mg per day (BID group; approximate average total exposure of 171 mg) of the GLP surfactant material (see Investigator's Brochure, Section 4.2.1) as compared to baseline and other treatments (e.g., no treatment, aerosol alone, or surfactant plus phenylephrine) (10, 11). In the ears of animals with AOM, treatment with 40 mg per day for 10 days of the GLP surfactant material (total exposure of 400 mg) as compared to propellant alone, was associated with a greater proportion of normal tympanometry findings, a lower percentage of culture positivity,

a lower severity of ME disease (occurrence of labyrinthitis) and a greater proportion of animals without ME fluid (i.e., dry ears) (2).

Results from these animal model studies demonstrate that single doses of 20 mg (given as 2 sprays per nostril [5 mg per spray]) and daily repeated exposures (either 20 mg once daily or 20 mg twice daily) resulting in total cumulative doses of up to 400 mg over 10 days (see Investigator's Brochure: Section 4.2.1) applied via intranasal MDI were safe and well-tolerated and the measured effects on ET POP were generally consistent across animal model studies (2, 9-11)

The clinical safety and tolerability of OP0201 manufactured by Novus Therapeutics, Inc. for human use is further guided by a limited amount of data from nine human cases . A total of eight cases were exposed to a different formulation, which was DPPC:CP 16:1 w/w see Investigator's Brochure: Section 5.1.1). One case was exposed to another different formulation comprised of DPPC:CP 200:1 see Investigator's Brochure: Section 5.1.1.

3.6.7 Blinding

Novus Therapeutics (or designee) will provide IMP in masked numbered kits that contain identically appearing canisters. Subjects and all site personnel will be blinded to the IMP assignment throughout the duration of the trial.

3.6.7.1 Unblinding

When necessary for the safety and proper treatment of the trial subject, the investigator can unblind the trial subject's treatment assignment to determine which treatment has been assigned and institute appropriate follow-up care. When possible, Novus Therapeutics Medical Safety Physician (or designee) should be notified prior to unblinding. The investigator should inform the Novus Therapeutics Medical Safety Physician (or designee) of the unblinding if there is no notification prior to the unblinding.

Emergency envelopes with randomization codes for specific kit IDs are provided to the site and the safety management by IMSB. The reason for breaking the code must be recorded in the trial subject's source documents.

3.6.8 Previous and concomitant medication

Previous medications used within 12 weeks prior to screening will be recorded. Any concomitant medications used during the study will be recorded starting from time of informed consent.

The decision to administer a prohibited medication is done with the safety of the trial subject as the primary consideration. When possible, Novus Therapeutics should be notified before the prohibited treatment or medication is administered.

The following medications are prohibited throughout the duration of the trial:

- Medications (either topically or systematically) for a condition related to ear or nose.
- Anti-cholinergic medications (eg, anti-histamine, anti-nausea)
- Any prescription drug (except oral contraceptives, see Section 3.6.9)
- Any over-the-counter (OTC) drugs including herbal supplements, vitamins, dietary supplements (except for the occasional use of paracetamol not exceeding 3 grams per day)

3.6.9 Pregnancy and Contraception Considerations

Definition of Female of Child-bearing Potential

For purposes of this study, a female will be considered of childbearing potential unless she is permanently sterilized (i.e., hysterectomy) or is naturally postmenopausal. Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman who is at least 40 years of age has experienced 12 months of amenorrhea without any other obvious pathological or physiological cause. Female subjects who have had a hysterectomy or are naturally postmenopausal will not be required to use contraceptives during the trial.

Pregnancy Considerations

All female subjects who are of child-bearing potential will be advised to avoid pregnancy and are required to use methods of contraception considered acceptable to Novus Therapeutics during the trial.

Acceptable Contraception

The investigator will review the contraception requirements for the trial with each subject.

The following methods of contraception with a Pearl Index lower than 1%, if properly used, are considered acceptable for use to avoid pregnancy during this study: oral hormonal contraception (‘pill’), dermal hormonal contraception, vaginal hormonal contraception (NuvaRing®), contraceptive plaster, long-acting injectable contraceptives, implants that release progesterone (Implanon®), tubal ligation (female sterilisation), intrauterine devices that release hormones (hormone spiral), double-barrier methods.

This means that the following are not regarded as safe: condom plus spermicide, simple barrier methods (vaginal pessaries, condom, female condoms), copper spirals, the rhythm method, basal temperature method, and the withdrawal method (coitus interruptus).

3.6.10 End of Trial

The end of trial date for each subject (i.e. completion of Visit 5/Day 9) will be recorded in the eCRF.

3.6.11 Continuation of treatment after the end of the clinical trial

This section does not apply for this trial.

3.7 Efficacy and safety variables

3.7.1 Safety variables

Otoscopy

Otoscopy will be performed to assess the appearance of the TM. The TM will be rated for the following variables:

1) Contour (normal, retracted, full, bulging, perforated),
2) Color (normal, partly red, completely red),
3) Fluid behind the TM (no/yes; if yes – yellow, translucent, red, blue or black)
4) Translucency (translucent, semi-opaque, opaque)
5) TEED classification (12, 13):
    - TEED 0 = normal otoscopy
    - TEED 1 = retraction and increased vascularization of manubrium and Schrapnell
    - TEED 2 = retraction and hyperemia of the entire eardrum
    - TEED 3 = fluid or blood in the middle ear
    - TEED 4 = perforated eardrum

Tympanometry

Tympanometry will be performed to assess TM mobility, ET function and middle ear function by measuring the amount of sound energy reflected back when a small probe is placed in the ear canal (14). The output of the right ear and left ear tympanometry will be a tympanogram tracing classified as follows (15):

- Type A
- Type B
- Type C

Nasal and Epipharynx Endoscopy

Nasal and epipharynx endoscopy will assess for normal or abnormal appearance. Abnormal appearance of nose and/or epipharynx will be documented and captured as applicable: swelling, redness, bleeding, rhinorrhoea (for the nose only) or, if applicable, other abnormal result that should be then specified.

At the screeing vist, as a part of the nasal endoscopy, the length of the nasal cavity (in cm) will be recorded separately for the left and right side.

12-Lead ECG

A 12-lead ECG will be performed at the screening visit to ensure that a trial subject does not have, in the investigator's clinical judgment, a clinically significant condition or a condition that may pose an unacceptable safety risk for study participation. Triplicate 12-lead ECGs will be performed at Day 1 (pre-dose, 150-215 min) and Day 8 (pre-dose, 150-215 min).

ECGs will be assessed by a central ECG reading center (ERT). An ERT cardiologist will review screening, pre- and post-treatment ECGs and discuss any abnormal findings with the investigator, as appropriate.

Physical Exam

Physical exam will be performed to assess any physical abnormalities. The body systems to assess are: general appearance, overall status of the skin, head, neck, trunk, eyes, heart and lungs (eg, breathing sounds), abdomen, extremities, lymph nodes.

Vital Signs

Systolic and diastolic blood pressure (mm Hg), pulse rate (beats/minute), respiration rate (breaths/minute) and body temperature (°C) should be taken after a subject has been at rest (seated) for at least 2 minutes. Blood pressure will be recorded using a sphygmomanometer. Pulse rate and respiration rate will be measured by counting over 30 seconds and multiplying by 2. Respiration rate may be taken concurrently with all ECGs.

Clinical Laboratory Tests

Clinical laboratory tests will be analyzed at the local clinical laboratory of the University Hospital Cologne.

Blood samples

Blood samples (non-fasting) should be collected using standard laboratory instructions and procedures provided by the local laboratory. These blood specimens will be analyzed for the following:

- Hematology: hemoglobin [g/dL], % hematocrit, red blood cell count (RBC), white blood cell count (WBC), % neutrophils, % lymphocytes, % monocytes, % basophils, % eosinophils, platelet count, partial thromboplastin time [sec], and % prothrombin time.
- Biochemistry: sodium, potassium, chloride, magnesium, phosphorus, calcium, creatinine, urea nitrogen, uric acid, total bilirubin, direct bilirubin, alkaline phosphatase (total), glutamic-oxaloacetic transaminase (GOT), glutamicpyruvic transaminase (GPT), gamma glutamyltransferase (GGT), albumin, total protein.

General urine analysis

pH, protein, ketones, bilirubin, blood (total Hb), nitrite, urobilinogen, specific gravity. If there are any abnormalities in the urinalysis that suggest the need for urine microscopy, then this should be performed and reported as a comment.

Urine Drug Screen

A urine drug screen will be performed by the local laboratory of the University of Cologne using a commercially available urine drug test kit obtained from möLab GmbH, Langenfeld, Germany. This urine drug screen will test for the following:

Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methamphetamines, methadone, opiates.

No analysis is intended for this screening variable.

3.7.2 Pharmacodynamic Assessments

Pharmacodynamics of the ET will be assessed using continuous tympanic impedance measures (5). The following measurements will be recorded:

1. ET opening pressure (ETOP) indicating the function of the ET
   Passive (phase 1 and phase 5) : only the first ETOP [mbar] measurement will be recorded for left and right ear, separately
   Active (phase 3): all ETOP [mbar] measurements will be recorded for left and right ear, separately

2. ET opening duration (ETOD) indicating the duration between tube opening and closing

   Passive (phase 1 and phase 5): only the first ETOD [s] measurement will be recorded for left and right ear, separately
   Active (phase 3): all ETOD [s] measurements will be recorded for left and right ear, separately

3. ET closing pressure (ETCP) indicating atmospheric pressure event that induces ET closure

   Passive (phase 1): only one ETCP [mbar] measurement will be recorded for left and right ear, separately
   Active (phase 3): No measurements will be recorded

4. ET opening frequency (ETOF) indicating number of ET opening within a given time interval and depends on the rate of pressure decrease and increase measurement for passive and active ET function:

   Passive (phase 1 and phase 5): number of openings in phase 1 and 5 [/min] will be recorded for left and right ear, separately
   Active (phase 3): number of openings in phase 3 [/min] will be recorded for left and right ear, separately

3.7.3 Exploratory Efficacy Assessment

An exploratory efficacy assessment of OP0201 compared to placebo to ascertain whether the IMP modulates ear pain during the hypobaric/hyperbaric atmospheric pressure chamber protocol is planned. During the conduct of the hypobaric/hyperbaric atmospheric pressure chamber protocol subjects will rate the worst pain they felt in the right ear, and separately in the left ear during phase 1, during phase 3 and during phase 5. The pain will be rated using a whole number Numeric Rating Scale (NRS) ranging from 0 (no pain) to 10 (worst pain imaginable).

3.7.4 Demographics and Baseline Characteristics

Demographic data (age, race, gender) will be collected at the screening visit.

Medical, surgical and ear history will be collected at screening visit.

3.7.5 Rationale for assessment procedures

The safety of subjects participating in this phase 1 human clinical trial is the most important consideration as they would not normally be expected to derive any therapeutic benefit. The primary objective of this trial is to evaluate the safety and tolerability of a single dose of 20 mg OP0201 compared to placebo in healthy adult volunteers utilizing standard safety assessments including PE, ENT exam (i.e., otoscopy, nasal and epipharynx endoscopy, tympanogram), vital signs, clinical laboratory tests, triplicate 12-lead ECG and AE monitoring.

The two active ingredients, DPPC and CP, in OP0201 are highly endogenous in mammalian tissue including those of the nasopharynx and respiratory system. Therefore, it is essentially impossible

to measure administered concentrations of OP0201 using traditional pharmacokinetic assessments. Hence, pharmacodynamics assessments (continuous tympanic impedance) have been included to help characterize pharmacological effects on the ET. This method has been established as a dynamic evaluation of the ET (6).

It has been reported that in healthy people ear pain can result from exposure to pressure fluctuations caused by compression and decompression manoeuvers in a pressure chamber (5). Thus, an ear pain assessment has been included to explore whether OP0201 compared to placebo modulates ear pain as a possible efficacy measure.

3.8 Data quality assurance

3.8.1 Monitoring

A representative on behalf of Novus Therapeutics, Inc., namely, CTC Cologne, will monitor the trial. The determination of the extent and nature of monitoring will be based on consideration such as the objective, purpose, design complexity, blinding, size and assessments in the trial. The monitor will perform 100% source data verification. Details about the extent of monitoring will be described in a separate monitoring manual.

3.8.2 Audits/Inspections

As part of quality assurance, the Sponsor has the right to audit the trial sites and any other institutions involved in the trial. The aim of an audit is to verify the validity, accuracy and completeness of data, to establish the credibility of the clinical trial, and to check whether the trial subject's rights and trial subject safety are being maintained. The Sponsor may assign these activities to persons otherwise not involved in the trial (auditors). These persons are allowed access to all trial documentation (especially the trial protocol, case report forms, trial subjects' medical records, drug accountability documentation, and trial-related correspondence).

Authorized representatives of Novus Therapeutics, Inc. or regulatory authority representatives may conduct on-site visits to review, audit, and copy trial-related documents under applicable data protection regulations. These representatives will meet with the investigator(s) and appropriate staff at mutually convenient times to discuss trial-related data and questions.

The Sponsor and the trial site involved undertake to support auditors and inspections by the competent authorities at all times and to allow the persons charged with these duties access to the necessary original documentation.

All persons conducting audits undertake all measures to keep all trial subject data and other trial data confidential.

3.9 Documentation

Clinical documentation relevant to the trial includes all records in any form (including but not limited to written, electronic, ENT records and scans) that describe or record the methods, conduct and/or results of the trial, the factors affecting the trial and the actions taken.

Data for this trial will be recorded in source documents that may include subject's medical records, hospital charts, clinic charts, the investigator's trial subject trial files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms.

The data will be entered online using an eCRF at the trial site via the Internet. Access to an eCRF will be provided to the trial site personnel as required. Plausibility checks are run during data entry, thereby detecting many discrepancies immediately. The CTC Cologne Data Management will conduct further checks for completeness and plausibility and will clarify any questions with the trial sites electronically via the trial software. These electronic queries have to be answered by the trial site without unreasonable delay. Further details will be specified in the data management manual.

The following information should be entered into the trial subject's medical record:

- Trial subject's name
- Trial subject's contact information
- The date that the subject entered the trial, subject number and subject IMP kit number
- Site will receive the randomization confirmation for each transaction. All notifications are to be maintained with the trial source documents
- The trial title and/or the protocol number of the trial and the name of Novus Therapeutics
- A statement that informed consent was obtained including the date. A statement that data protection consent, or other country and local trial subject privacy required documentation for this trial has been obtained (including the date).
- Reasons for screen failure
- Dates of all trial visits
- Trial procedures and assessments (e.g., ECG, labs, otoscopy, TEED, tympanogram).
- All concomitant medications
- All concurrent procedures and non-medication treatments
- Occurrence and status of any adverse events (including onset date, assessments of severity, seriousness, and relationship with IMP, treatment instigated, action taken regarding IMP, outcome, and stop date).

- The date the subject exited the trial, and a notation as to whether the subject completed the trial or reason for early exit.
- The results of protocol-required laboratory tests performed by the site (e.g., results of urine pregnancy tests).
- Trial subject's medical, surgical, and ear histories (including demographics)
- For females, documentation of non-childbearing potential or results of pregnancy test and documentation of trial subject's stated birth control method (if applicable)
- Physical examination and vital sign results
- All trial subject questionnaires (ear pain assessments)
- Source notes should also include any trial subject counseling/education (when applicable) permissible medications/treatments, prohibited medications/treatments, and timing in relation to scheduled trial visits and trial compliance.

3.9.1 Data management

An electronic data capture (EDC) system will be used for this trial. The IT infrastructure and data management staff will be supplied by the CTC Cologne. The trial database will be developed and validated before data entry based on standard operating procedures at the CTC Cologne. The data management system is based on commercial trial software and stores the data in a database. All changes made to the data are documented in an audit trail. The trial software has a user and role concept that can be adjusted on a trial-specific basis. The database is integrated into a general IT infrastructure and safety concept with a firewall and backup system. The data are backed up daily. After completion and cleaning of data, the database is locked and the data exported for statistical analysis.

The data will be entered online at the trial sites via the Internet. Plausibility checks are run during data entry, thereby detecting many discrepancies immediately. The CTC Cologne Data Management will conduct further checks for completeness and plausibility and will clarify any questions with the trial sites electronically via the trial software. These electronic queries have to be answered by the trial site without unreasonable delay. Further details will be specified in the data management manual.

3.9.2 Archiving

All trial related correspondence, patient records, consent forms, patient privacy documentation, records of the distribution and use of all investigational products, and copies of case report forms should be archived for at least 10 years in accordance with §13 (10) of the GCP-V (German legal implementation of ICH-GCP regulation). Trial site shall ensure archiving and retention of essential

trial documents (e.g. ISF, subject files and source data), after the termination or discontinuation of the trial for at least 10 years unless longer times are foreseen by local regulatory requirements.

For countries falling within the scope of the ICH guidelines, the Sponsor-specific essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.

Novus Therapeutics, Inc. requires that it will be notified in writing if the investigator wishes to relinquish ownership of the data so that mutually agreed-upon arrangements can be made for transfer of ownership to a suitably qualified, responsible person.

4 Ethical and regulatory aspects

4.1 Independent ethics committee

The clinical trial will not be started before approval by the competent local ethics committee.

4.2 Ethical basis for the clinical trial

The present trial protocol and any amendments are prepared in accordance with the current version of the Declaration of Helsinki.

4.2.1 Legislation and guidelines used for preparation

The present clinical trial will be conducted in accordance with the published principles of the guidelines for Good Clinical Practice (ICH-GCP) and applicable legislation (especially the Federal Drug Law [AMG] and the GCP-V). These principles cover, amongst other aspects, ethics committee procedures, the obtaining of informed consent from trial subjects, adherence to the trial protocol, administrative documentation, documentation regarding the IMP, data collection, trial subjects' medical records (source documents), documentation and reporting of adverse events (AEs), preparation for inspections and audits, and the archiving of trial documentation. All investigators and other staff directly concerned with the trial will be informed that domestic and foreign supervisory bodies, the competent federal authorities and authorised representatives of the Sponsor have the right to review trial documentation and the trial subjects' medical records at any time.

4.3 Notification of the authorities, approval and registration

Before the start of the clinical trial, all necessary documentation will be submitted to the competent supreme federal authority for approval (Federal Institute for Drugs and Medical Products, Bundesinstitut für Arzneimittel und Medizinprodukte [BfArM]). The state authorities in the federal state in which the trial will be conducted will also be notified. Further, competent supreme federal authority, local authorities and ethics committee will be informed about the end of trial .

4.4 Obtaining informed consent from trial subjects

Written informed consent is to be obtained from each trial subject prior to any trial-related activities or procedures in the trial. Together with the consent to take part in the trial, the subject must also agree to representatives of the Sponsor (e.g. monitors or auditors) or the competent supervisory or federal authorities having access to the data recorded within the framework of the clinical trial. The subject will be informed of the potential benefit and possible side effects of the IMP and placebo, and of the need and reasons to conduct a placebo-controlled clinical trial. It must be clear to subjects that he or she can withdraw his/her consent at any time without giving reasons and without jeopardizing his/her further course of treatment. The subjects have sufficient time for consideration prior to giving their consent to participate in the trial.

The originally signed consent form is archived in the investigator site file. Trial subjects receive copies of the written information sheet, the confirmation of insurance with conditions, and the signed informed consent form.

4.5 Insurance of trial subjects

All trial subjects enrolled are insured in accordance with § 40 AMG (German Medicinal Law)

[redacted]

4.6 Data protection

A report of the results of this trial may be published or sent to the appropriate health authorities in any country in which the IMP may ultimately be marketed, but the trial subject’s name will not be disclosed in these documents. The trial subject's name may only be disclosed to Novus Therapeutics’ assigned auditor, who is obliged to confidentiality, or governing health authorities (e.g., US FDA, European Medicines Agency) if they inspect the trial site. Appropriate precautions will be taken to maintain confidentiality of medical records and personal information.

Data protection consent and other documentation in accordance with the relevant country and local privacy requirements (where applicable) is to be obtained from each trial subject prior to enrollment into the trial in accordance with the applicable privacy requirements (e.g., German Data Protection Law, European Union General Data Protection Regulation 2016/679).

Subjects will be informed that their pseudonymised data will be passed on in accordance with provisions for documentation and notification pursuant to § 12 and § 13 of the GCP Regulations to the recipients described there.

5 Statistical methods and sample size calculation

The analysis is descriptive, significance level is not adjusted for multiple testing. A p-value ≤ 0.05 is defined as significant for pharmacodynamic and efficacy assessments, demographic and screening characteristics. Confidence intervals will be calculated at the 95%-level.

Missing values will not be replaced, but drop-outs may be replaced with additionally randomized trial subjects to ensure N=16 subjects for the PP population.

5.1 Statistical and analytical plan

The analysis of trial data will be specified elaborately in a separate statistical analysis plan (SAP).

5.1.1 Analysis populations

The following populations will be used for analysis:

The safety population will consist of all randomized trial subjects who received at least one spray of IMP in either nare. In safety data analyses, subjects will be analysed according to actual IMP received, regardless of randomization assignment.

The per-protocol (PP) population consists of all randomized subjects who received the two complete doses (4 sprays to each nare) of IMP at Day 1 and Day 8 . Valid measures of ETOP and ETOD in passive phases 1 and 5 need to be collected for both ears and for all three measurement times at visits 2 and 4. Excluded are subjects with major protocol deviations (determined prior to database lock and unblinding of the data).

5.1.2 Description of trial subject groups

For all variables and analysis populations summary statistics will be calculated, grouped by treatment, period and measurement time, where applicable. If variables are measured before and after treatment at the same visit, the difference will be calculated - if appropriate - for each timepoint for visit 2 and separately for visit 4, and summary statistics will also be given.

Descriptive statistics include for quantitative variables the number of subjects, mean, standard deviation, median, median confidence interval, quartiles, minimum, maximum; and for qualitative variables: number of subjects, absolute and relative response frequencies. Confidence intervals may be calculated if appropriate.

5.1.3 Primary objectives

Primary analysis is the evaluation of the safety variables in the safety population.

Incidence of AEs will be tabulated by primary system organ class (SOC) and by preferred term within each primary SOC, according to MedDRA code. The severity of the AEs will also be summarized. AE and SAE will be listed by subject, treatment and period.

Analyses for single parameters will be specified in more detail in the SAP.

5.1.4 Secondary objectives

Pharmacodynamics of the ET and ear pain will be analysed in the safety population and additionally in the PP population. Treatment comparisons will be made according to Grizzle's two-stage approach of the cross-over design by two-sided tests, where appropriate (16). The applied test for comparison within the Grizzle's two-stage concept is the Wilcoxon rank-sum test. Non-parametric testing is planned for the main cross-over analysis because the determination of the distribution of variables is difficult with respect to the small sample size and the potential for outliers. In a sensitivity analysis, a parametric cross-over analysis will be performed. Tests will be carried out without covariates. Missing data will not be imputed. Exact Hodges-Lehmann confidence intervals will be calculated for estimates of treatment effects. Appropriate sequence-by-period plots will be produced to present tympanic impedance variables.

5.1.5 Subgroup analyses

If sufficient numbers of subjects are met, subgroup analyses by stratification factors are planned. Details will be specified in the SAP.

5.1.6 Interim analysis

An interim analysis is not planned in this trial.

5.2 Sample size calculation

Using a one-sample t-test with a significance level of 0.05 (2-sided) and a power of 0.8, a sample size of 14.3 is required in order to detect a effect size (treatment difference standardized by

standard deviation) of 0.8 (17). Since the ear pain and tympanic impedance variables will be analyzed primarily with a non-parametric method (whose asymptotic relative efficiency to the t-test under assumption of normal distribution is 0.955 (18)), the final sample size is adjusted to 16 (=ceiling(14.3/0.955)) in total and 8 per sequence.

6 Safety

6.1 Definitions of adverse events and adverse drug reactions

6.1.1 Adverse event

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

AEs will be assessed, documented, and recorded throughout the trial. AEs will be collected from the time after informed consent is signed until the end of trial for each subject (Day 9, trial exit).

At each visit, the investigator will begin by querying for adverse events by asking each trial subject a general, non-directed question such as “How have you been feeling since the last visit?” Directed questioning and examination will then be done as appropriate. All reported adverse events will be documented on the appropriate eCRF.

If, after obtaining the trial subject’s informed consent but prior to administration of trial product/treatment, a medical condition is identified via a trial entry protocol assessment or procedure (e.g., screening ECG test) which, based on the Investigator’s judgment (and in consultation with the trial subject), is determined to be a pre-existing condition (i.e., a condition existing prior to the time of the trial subject’s informed consent), it should be documented on the Medical History eCRF. Otherwise, the condition should be reported as a pre-treatment AE.

6.1.2 Adverse drug reaction

An adverse drug reaction (ADR) is any noxious and unintended response to an IMP related to any dose with at least a reasonably possible causal relationship with the IMP.

6.1.3 Serious adverse events or serious adverse drug reactions (SAE/SADRs)

A SAE/SADR is any adverse event that results in any of the following outcomes:

- Death,
- A life-threatening adverse event,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant disability/incapacity,
- Or a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event, when based upon appropriate medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Any SAE must be reported immediately but no later than 24 hours after becoming aware of the SAE by the investigator to Sponsor or authorized representative. SAEs must be reported directly to the Sponsor authorized representative, namely, CTC Cologne as listed on the Trial Contacts Page and recorded on the SAE Form. All subjects with SAE/SADR must be followed up and their outcomes reported. In the case that the outcome is death, SAEs must be directly reported to the responsible ethics committee and competent authority according to §12 (6) GCP-V. The investigator must supply Novus Therapeutics, CTCC, the responsible ethics commitee and responsible competent authority with any additional requested information (e.g., autopsy reports, discharge summaries).

6.1.4 Unexpected adverse drug reaction (UADR)

An UADR is an ADR which, the nature or severity of which is not consistent with the applicable product information available for the IMP. Further information is described in the Investigator's Brochure Section 6.3.1.

All SAEs that are drug-related and unexpected (not listed as treatment-related in the current Investigator's Brochure) must be reported to the competent authority, responsible ethics committee and/ or other bodies according to local regulations.

6.2 Documentation and follow-up of AEs and ADRs

The Sponsor ensures that all persons involved in the treatment of trial subjects are adequately informed of the responsibilities and actions required when AEs and/or ADRs occur. AEs and ADRs will be documented in the trial subject's medical records and in the eCRF.

For all AEs and ADRs the following information will be documented in the source documentation and the eCRF:

- AE verbatim terminology and if applicable location (e.g., left ear pain)
- Onset and resolution date

- Severity
- Causal relationship with IMP
- Seriousness
- Action taken with IMP and other measures taken
- Outcome

Regardless of whether a causal relationship between the AE and the IMP is suspected, trial subjects who develop AEs must be monitored within the trial period until all symptoms have been subsided, pathological laboratory values have returned to pre-event levels, a plausible explanation is found for the AE/ADR, or the trial subject has died.

Adverse reactions reported for other products that contain both DPPC and CP are discussed in Section 6.3.2 of the Investigator's Brochure. Adverse reactions reported for intranasal HFA-134a (placebo) are discussed in Section 6.3.3 of the Investigator's Brochure.

For the procedure of SAE documentation and reporting see section 6.2.3 and 6.2.4, respectively.

6.2.1 Severity of the AE

A clinical determination will be made of the intensity of an AE. The severity assessment for a clinical AE must be completed using the following definition as guidelines:

- Mild - Awareness of sign or symptom, but easily tolerated
- Moderate - Discomfort enough to cause interference with usual activity
- Severe - Incapacitating with inability to work or do usual activity

6.2.2 Causal relationship between AE and IMP

The investigator is obligated to assess causal relationship (related or not related) between study drug and each AE/SAE.

Related: A causal relationship is present if a determination is made that there is a reasonable possibility that the AE/SAE may have been caused by the study drug. A reasonable possibility of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

Not related: There is not a reasonable possibility that the AE may be related to the study drugs.

The investigator will use clinical judgment to determine the relationship. The investigator will also consult the Investigator's Brochure to aid his/her assessment. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration should also be considered and investigated.

For each AE/SAE, the investigator must document in the source documents that he/she has reviewed the AE/SAE and has provided an assessment of causality. There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to CTCC. However, it is important that the investigator makes an assessment of causality for every event before the initial transmission of the SAE data to CTCC.

The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

6.2.3 Documentation and reporting of SAE and pregnancy

Regardless of the assumed causal relationship, every SAE that occurs within the trial period must be documented in the source documentation and in the appropriate part of the eCRF. Additionally, all SAEs must be reported to CTCC by using the study specific SAE report form. SAEs after the reporting period must be reported if the investigator suspects a causal relationship between the IMP and the SAE.

Pregnancies must also be documented in the source documentation,in the eCRF (on separate pregnancy reporting page and on the pregnancy outcome form) and additionally on the study specific pregnancy form and reported to CTC Cologne.

Details of Safety Management are defined in a trial-specific Safety Manual, which describes information and communication pathways, important timeframes for reporting, interfaces and clarification of responsibilities and national statutory requirements.

<u>Pregnancy</u>

The investigator will inform CTC Cologne without delay about any pregnancy that occurs within the trial period, i.e. within 24 hours of being made aware of such. This will be documented on a separate "Pregnancy Report Form I". Pregnant subjects should not receive further study treatment. The pregnant trial subject will be asked to give separate informed consent for pregnancy follow up after the completion of the trial. The investigator must document the outcome of the pregnancy and provide the documentation to the Sponsor. A separate "Pregnancy Report Form II" should be sent to CTC Cologne in a timely fashion after becoming aware of the pregnancy outcome. Both parents

are asked to give separate informed consent for child follow up. For live births, the health of the child should be monitored until the age of one month.

If an SAE/SUSAR of mother or child occurs in the course of pregnancy or delivery, it has to be reported according to statutory requirements.

6.2.4 SAE reporting by the investigator to the Sponsor

In the event of a SAE, the investigator must:

1. Inform the CTC Cologne, which is assigned with Safety-Management duties by the Sponsor, without delay (at latest 24 hours after being aware of the SAE) by sending the SAE report form, via Fax.

   [redacted]

   [redacted]

2. Obtain and maintain in his/her files all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the subject.

3. Provide Sponsor with a complete, written description of adverse event(s) on the SAE Form describing the event chronologically, including any treatment given (e.g., medications administered, procedures performed) for the adverse event(s). Summarize relevant clinical information about the event: signs, symptoms, diagnosis, was or clinical course and relevant clinical laboratory tests, etc. Include any additional or alternative explanation(s) for the causality which includes a statement as to whether the event was or was not related to the use of the investigational drug.

Each SAE collected in the trial period must be followed up until one of the following occurs:

- The SAE is no longer serious.
- The subject dies.

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs/SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up.

The recurrence, deterioration or ending of an existing SAE but also each correction of an SAE-report will be documented as a follow-up report and will be reported to the Sponsor or its designee (i.e. CTC Cologne) immediately.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the Sponsor.

6.2.5 Assessment of Serious Adverse Event by Sponsor

All SAEs are assessed by the Sponsor and PI with regard to seriousness and causality. The expectedness assessment will be done by Sponsor.

If an AE is “serious”, “related” and “unexpected”, the criteria for an expedited report (Suspected Unexpected Serious Adverse Reaction, SUSAR) are fulfilled.

When necessary for the safety and proper treatment of the subject, the investigator can unblind the subject’s treatment assignment to determine which treatment has been assigned and institute appropriate follow-up care. When possible, the Novus Therapeutics Medical Safety Physician (or designee, namely CTCC) should be notified prior to unblinding IMP. The investigator should inform the Novus Therapeutics Medical Safety Physician (or designee, CTCC) of the unblinding if there is no notification prior to the unblinding.

6.2.6 Notification of ethics committee and competent supreme federal authority

Adverse event reporting, including suspected unexpected serious adverse reactions, will be carried out in accordance with applicable local regulations (GCP-V §13 (1-4). CTCC will provide regulatory authorities with safety updates/reports (e.g. DSUR; GCP-V §13 (6)) according to local requirements, including suspected and unexpected serious adverse reactions, where relevant.

6.2.7 Notification of study staff and study participants

Safety-relevant results from ongoing studies will be communicated by the Novus Medical Safety Physician (or designee) to the Study Safety Physician(s) (e.g. at the CRO CTCC), the Principal Investigator(s) and CRO Project Managers. Investigators and the CRO Project Manager are responsible for ensuring new safety information is provided to appropriate study staff in a timely manner. Investigators will inform study participants of any new safety findings in a timely manner.

The Informed Consent and the Investigator's Brochure may be updated with new safety information as appropriate.

6.2.8 Informing the Data Monitoring Committee

A DMC is not planned in this trial.

6.2.9 Known side effects caused by pressure differential during pressure chamber measurement protocol

In very rare cases, injuries may result from pressure variations during compression or decompression (barotrauma) during the pressure chamber protocol including, for example, reddening of the tympanic membrane, bursting of a blood vessel on/near the tympanic membrane or accumulation of fluid behind the tympanic membrane. In rare cases, subjects can experience temporary ear pain, even if they do not have any prior history of ear pain when experiencing atmospheric pressure changes (e.g. during take-off or landing in an airplane; scuba diving). Serious ear injuries do not happen suddenly and, if they occur, appear slowly during the pressure variations and are manifested as growing ear pain prior to ear injury. Some subjects may experience anxiety or claustrophobia while in the chamber.

The risk from pressure chamber exposure for the subject is minimized because the chamber protocol does not expose the subject to large atmospheric pressure changes for long periods of time. Subjects will undergo two pressure profile simulations representing (1) an airplane ascent and airplane descent and (2) a scuba dive and return to sea level. Subjects are exposed for a total of 4.5 minutes to atmospheric pressure changes per each pressure chamber assessment. At each of the Day 1 and Day 8 visits, the exposure will be a total of 13.5 minutes over a 4-6-hour period (total exposure 27 minutes across both days). Limiting the total daily time in the chamber reduces the risk for decompression illness, hypoxia, barotrauma or other potential hazards from excessive decompression.

During the 4.5-minute chamber protocol, the investigator is constantly monitoring the pressure and observing the study participant visually (through the chamber window) and is able to speak to and hear from the study participant via an internal speaker system embedded in the chamber. If at any time the study participant appears to be experiencing excessive ear pain, anxiety, claustrophobia or for any other reason in the Investigator's judgement, the pressure chamber protocol will be stopped immediately, the door of the chamber will be opened, and the study participant will be removed from the chamber.

7 Use of trial findings and publication

7.1 Reports

7.1.1 Interim reports

No interim report is planned for this trial.

7.1.2 Final report

The competent authority and ethics committee will be informed within 90 days that the trial has officially ended.

Within one year of the completion of the trial, the competent federal authority and the ethics committee will be supplied with a full final report on the clinical trial according to ICH E3 describing the results according to §13 (9) GCP-V.

7.2 Publication

The Sponsor will comply with the requirements for publication of study results in accordance with standard editorial and ethical practice. Novus Therapeutics, Inc., as the Sponsor, has proprietary interest in the trial and thus will be involved in reviewing, at a minimum, any abstract or manuscript prior to submission in order to allow the Sponsor to protect proprietary information and to provide comments. For this study, authorship and abstracts or manuscripts composition will reflect joint cooperation between the investigator and Novus Therapeutics, Inc. personnel. Authorship will be 1) established prior to the writing of abstracts or manuscripts, 2) determined by mutual agreement and 3) in line with International Committee of Medical Journal Editors authorship requirements.

7.3 Amendments to the trial protocol

The investigator must not implement any deviation from or changes to the protocol without approval by Novus Therapeutics and prior review and documented approval/favorable opinion from the IEC of a protocol amendment, except where necessary to eliminate immediate hazards to study subjects.

Amendments made in accordance with § 10 Secs. 1 and 4 GCP Regulations that require approval are submitted to the ethics committee and the competent supreme federal authority and will not be implemented until approved.

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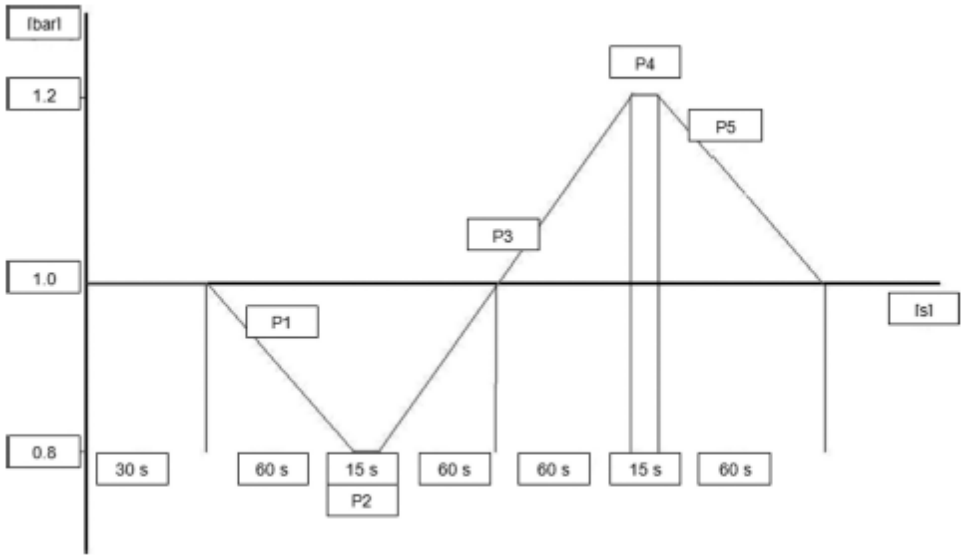
9 Attachments

9.1 Hyperbaric/hypobaric pressure chamber measurement

Pharmacodynamics is based on an exploratory analysis of ET function (by continuous tympanic impedance) of subjects whilst being exposed to an (semi)auto-modulated atmospheric pressure profile ranging from 0.8 bar to 1.2 bar in a single-person hypobaric/hyperbaric pressure chamber (Haux Life Support, Karsbad, Germany). The resulting pressure profile consists of five phases which are (Figure 2):

- Phase 1 (P1): decompression atmospheric 1 bar to 0.8 bar
- Phase 2 (P2): stationary at 0.8 bar (for 15 sec)
- Phase 3 (P3): compression from 0.8 bar to 1.2 bar
- Phase 4 (P4): stationary at 1.2 bar (for 15 sec)
- Phase 5 (P5): decompression back to atmospheric 1 bar

Figure 2: Standard pressure profile during continuous tympanic impedance measurement



Adpated from Meyer and colleagues (5)

9.2 Ear Pain Assessment (Example Page)

EAR PAIN ASSESSMENT #1

Think about the pain you felt in your left ear and separately in your right ear during the decompression phase in the hypobaric/hyperbaric pressure chamber.

Please mark one answer for each question by marking (☑ or ☒) in the box.

Left Ear Question: On a scale of 0 to 10, with 0 being no pain at all and 10 being the **worst** pain imaginable, how would you rate your **worst** level of pain in your **LEFT** ear?

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7	8	9	10

No Pain **Worst Pain Imaginable**

Right Ear Question: On a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your **worst** level of pain in your **RIGHT** ear?

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7	8	9	10

No Pain **Worst Pain Imaginable**

9.3 TEED Classification

TEED classification (12, 13):

- TEED 0 = normal otoscopy
- TEED 1 = retraction and increased vascularization of manubrium and Schrapnell
- TEED 2 = retraction and hyperemia of the entire eardrum
- TEED 3 = fluid or blood in the middle ear
- TEED 4 = perforated eardrum

Exemplary images of changes to the TM are shown for assigning classifications 1, 2, 3 and 4 according to TEED (12) adapted by Edmonds (13)