Study Title	EVALUATION OF SAFETY OF REPEATED DOSES OF OP0201 METERED DOSE INHALER COMPARED TO PLACEBO IN HEALTHY ADULT VOLUNTEERS
NCT Number	NCT03748758
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Document Date	09 October 2018

TITLE PAGE

Protocol Title:

Evaluation of safety of repeated doses of OP0201 metered dose inhaler compared to placebo in healthy adult volunteers.

Protocol Number: OP0201-C-002

Amendment Number: Amendment 1

Product: OP0201 metered dose inhaler

Study Phase: 1

Sponsor Name: Novus Therapeutics, Inc.

Legal Registered Address: 19900 MacArthur, Suite 550, Irvine, California 92612, United States (US)

Regulatory Agency Identifying Number: IND 106778

Date of Protocol: 09 October 2018

Sponsor Signatory:

I have read this protocol in its entirety and agree to conduct the study accordingly:

[redacted] **Date**

[redacted]

Medical Monitor name and contact information can be found in Appendix 2.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

Table 1 Document History

Document	Date	Substantial	Region
Amendment 1	09 October 2018	Yes	US
Original Protocol	17 July 2018	-	-

Amendment 1 (09 October 2018)

Overall Rationale for the Amendment:

During creation of the electronic case report forms (eCRFs) on otoscopy assessment, a discrepancy was noted in the way the assessments are described in the eCRF versus the protocol. It was thus decided to amend the description in the protocol to match the eCRF. Additionally, during the eCRF creation, updates to the section on nasal and epipharynx endoscopy, and audiology pure tone hearing test was also updated in the protocol. Moreover, discussions with IQVIA biostatisticians and the study center led to the update of the randomization procedure, the number of days residency in the study center and clarity on assessments to be performed in the case of early termination of the study. Due to logistical reasons, the telephonic study exit visit at Day 30 was removed and Day 21 will now be the study exit visit.

Table 2 Description of Changes in Amendment

Section # and Name	Description of Change	Brief Rationale
Synopsis – Overall design, Section 4.1 Study Design and Section 6.3.1 Study Treatment Kit	Removal of stratification by gender.	Per the protocol, randomization is to be stratified by gender within each of the 2 cohorts (Cohorts A and B) and subjects are to be randomized in 4:1 ratio to active:placebo within each cohort. After further discussion with IQVIA biostatisticians and the Sponsor, it was agreed that gender stratification within a 4:1 randomization scheme would be problematic. Thus, gender stratification is removed from the protocol.
Section 1.3 Schedule of Activities	Addition of early termination (EaT) assessments to align with the Day 21 study exit visit.	Per the protocol, no assessments are specified for cases where a subject has been treated but wants to discontinue early from the study. IQVIA, in consultation with the Sponsor, discussed and agreed that as a standard, when subjects discontinue early, if possible, they should complete all study procedures required at the stuidy exit vist. Thus, the protocol has been amended to reflect this.

Section # and Name	Description of Change	Brief Rationale
Synopsis – Overall design, Section 1.2 Schema, Section 1.3 Schedule of Activities and Section 4.1 Study Design	Removal of the option for subjects to leave the study center on the evening of Day 14 after all the assessments have been completed.	Previously, the protocol gave the option for subjects to leave the study center on Day 14 after all the assessments have been completed. However, in consultation with the study center it was decided that the timing of the last dose administration on Day 14 (23:00) was too late and that most subjects would opt to stay the night and leave the study center on the morning of Day 15. The protocol has been amended to reflect this.
Synopsis – Overall design, Section 1.2 Schema, Section 1.3 Schedule of Activities and Section 4.1 Study Design	The telephonic study exit visit at Day 30 was removed and Day 21 will now be the study exit visit.	Due to logistical reasons, and the fact that the drug is short acting with no known or projected long-term adverse events, the follow-up period was shortened.
Synopsis, Number of Subjects	Number of subject to be screened changed from 35 to 60.	The study center will use a 1:1 screen fail ratio. Therefore, up to 60 subjects will be screened to enroll 30.
Section 1.3 Schedule of Activities and Section 6.1 Study Treatment(s) Administered	Schedule of dose administration changed to every 7 hours, ie, a morning dose at 9:00 am, an afternoon dose at 16:00 pm and an evening dose at 23:00 pm.	Due to logistical reasons at the study center.
Section 1.3 Schedule of Activities and Section 8.2.7 Nasal and Epipharynx Endoscopy	Amend the protocol to allow for all pre-dose ear, nose and throat (ENT) procedures to be completed on Day -1 instead of Day 1. Removal of footnote for performing scoping within 4 hours of pre-dose on all other days so that there isn't a time constraint.	Due to logistical reasons at the study center as there will not be enough time to complete the ENT procedures on this day within window.
Synopsis, Objectives and Endpoints, Section 3.0	Plasma replaced with serum.	The table of endpoints was in error and listed plasma istead of serum as the detection matrix for biomarker analysis.
Section 5.2 Exclusion Criteria	Addition of exclusion criteria: "History of drug abuse or positive drug screening test at Screening or Day -1 prior to randomization".	Previously a urine drug screen was included in the Schedule of Activities, but no corresponding exclusion criteria existed.
Section 8.2.5 Otoscopy	Update to otoscopy assessments by removing mention of translucency and addition of colors to the section	Based on the update to the eCRFs regarding the otoscopy assessment, it was decided to update Section 8.2.5 of the protocol so that it matches the

Section # and Name	Description of Change	Brief Rationale
	preregarding the color of fluid behind the tympanic membrane.	way this procedure will be conducted and how the data will be collected.
Section 8.2.7 Nasal and Epipharynx Endoscopy	Updated assessments to be made when abnormal endoscopy is reported; additional criteria includes: swelling, redness, bleeding, rhinorrheoea (nose only) and other.	Based on the update to the eCRFs regarding the nasal and epipharynx endoscopy assessment, it was decided to update Section 8.2.7 of the protocol so that it matches the way this procedure will be conducted and how the data will be collected.
Section 8.2.9 Audiology Pure Tone Hearing Test and Appendix 10 Audiology Testing Template	Section 8.2.9 updated to refer to new Audiology Testing Template in Appendix 10	The Audiology Testing Template provided by the study center was used to develop the eCRF page. The template is attached as a sample source for reference.
Appendix 3, Table 8	Duplication of potassium and urea/blood urea nitrogen corrected.	Study center noticed duplication of potassium and urea in Table 8.

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1.0 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title:

Evaluation of safety of repeated doses of OP0201 metered dose inhaler compared to placebo in healthy adult volunteers

Rationale:

OP0201 metered dose inhaler (MDI) (referred herein simply as OP0201) is a novel, intranasal drug device surfactant product being developed by Novus Therapeutics, Inc., for the treatment and prevention of otitis media (OM). To date, there is no drug product that has been approved to treat or prevent OM.

OP0201 is comprised of a 20:1 fixed combination of dipalmitoylphosphatidylcholine (DPPC; a phospholipid surfactant) and cholesteryl palmitate (CP; a neutral phospholipid spreading agent) suspended in propellant (HFA 134a). There are no other ingredients (ie, no excipients, no fillers) in the formulation other than the active ingredients and the propellant. None of these ingredients contain any animal or human derivatives. Both DPPC and CP are highly endogenous surfactants in humans, including the Eustachian tube (ET), nasopharynx and respiratory system. The drug product will be delivered as a local treatment through each nostril using a metered dose inhalation device. It will be sprayed directly backwards into each nostril by way of the anterior nostrils (not upwards towards the osteomeatal opening to the maxillary and ethmoid sinuses as other common intranasal products) towards the lateral wall of the nasal cavity so that the usual nasal mucociliary clearance pathway can facilitate delivery of the drug to the ET.

This Phase 1 safety study is designed to evaluate the safety and tolerability of daily intranasal OP0201 use for 14 consecutive days in 30 healthy adults. The study will be randomized, double-blind, placebo-controlled, parallel-group, and includes a dose-escalation cohort design with evaluation of 30 mg per day (Cohort A) and 60 mg per day (Cohort B) of OP0201.

For more information about OP0201, please refer to the most recent Investigator's Brochure (IB).

Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of OP0201 30 mg per day (given as 2 sprays per nostril 3 times a day [TID] for 14 consecutive days) and OP0201 60 mg per day (given as 4 sprays per nostril TID for 14 consecutive days) compared to 0 mg placebo (HFA-134a only) administered in a similar parallel-group fashion TID for 14 consecutive days in healthy adult volunteers.	• Adverse events (AEs), otoscopy, tympanometry, nasal and epipharynx endoscopy, University of Pennsylvania Smell Inventory Test (UPSIT) olfactory test, audiology pure tone hearing test, triplicate 12-lead electrocardiogram (ECG), physical examination, vital signs, and clinical laboratory tests.

Secondary/Exploratory	
• To evaluate if any systemic exposure of DPPC can be detected in serum at levels higher than endogenous DPPC levels in serum in up to 15 healthy adult volunteers who will be evaluated in the Cohort B dose (OP0210 60 mg per day) versus placebo. • To evaluate if any systemic exposure of CP can be detected in serum at levels higher than endogenous CP levels in serum in up to 15 healthy adult volunteers who will be evaluated in the Cohort B dose (OP0210 60 mg per day) versus placebo.	• Maximum baseline adjusted serum concentration (C_{max}) of DPPC on Day 14. • Time to maximum concentration (t_{max}) of DPPC on Day 14. • Maximum baseline adjusted serum concentration (C_{max}) of CP on Day 14. • Time to maximum concentration (t_{max}) of CP on Day 14. • Additional parameters may be calculated, if appropriate.

Overall Design:

This is a randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study in healthy volunteers to evaluate the safety and tolerability of OP0201 administered intranasally TID using a MDI compared to a matching placebo (HFA 134a minus active ingredients) administered intranasally TID using a MDI for 14 consecutive days. The plan is to enroll up to 30 subjects at 1 study center in the United States. Two dose cohorts are planned (Cohort A and Cohort B; N=15 per cohort). Within each cohort, subjects will be randomized in a 4:1 ratio to receive either OP0201 or placebo. After all subjects in Cohort A have completed the Day 14 visit, a Safety Review Committee (SRC) will review all the blinded safety data to determine whether the doses administered are safe and well tolerated. The SRC will make a recommendation to escalate to the next cohort (Cohort B) or not.

Potential subjects will be screened for eligibility during a screening period (Days -28 to -1). Eligible healthy volunteers will be admitted to the study center on Day -1 and be randomized on Day -1. Subjects will remain resident at the study center until after all the Day 15 assessments have been completed, with a study exit visit at Day 21.

Study treatment will be administered to the subjects by the study center staff TID for 14 days while resident at the study center.

The estimated duration of participation in the study for each subject is up to 54 days: Screening (Days -28 to -1), treatment (Day 1), 16-day residency (Days -1 to 15 with safety assessments at Days 4, 8, 14, and 15), study exit (Day 21 [+5 days]). No efficacy assessments will be performed for this study.

Number of Investigators and Study Centers:

Approximately 1 Investigator and study center are expected to participate in this study.

Number of Subjects:

Approximately 60 subjects will be screened to achieve 30 subjects randomly assigned to study treatment and 30 evaluable subjects for a total of 15 evaluable subjects per treatment group. Additional subjects may be screened to ensure a total of 15 evaluable subjects per treatment group. Subjects who are discontinued from the study may be replaced based on the Sponsor's discretion.

Treatment Groups and Duration:

Cohort	Dose Regimen	Cohort Dose Allocation		Total Subjects in Each Cohort	Total Subjects Per Dose Group
		Per Day Total Dose (mg)	Cumulative Total Dose (mg)		
A	**2 sprays per nostril TID × 14 days**	OP0201 (30 mg)[a] Placebo (0 mg)	OP0201 (420 mg)[a] Placebo (0 mg)	15	N=12 (OP0201) N=3 (Placebo)
B	**4 sprays per nostril TID × 14 days**	OP0201 (60 mg)[a] Placebo (0 mg)	OP0201 (840 mg)[a] Placebo (0 mg)	15	N=12 (OP0201) N=3 (Placebo)

a 2.5 mg per spray
N = number of subjects

Statistical Methods:

Sample Size Determination

The sample size is not based on statistical considerations, but is typical for studies of this nature, and is considered adequate to characterize the distribution of the planned endpoints.

There will be a total of 30 subjects; 2 dose cohorts of 15 subjects each. Subjects will be randomized in 4:1 (OP0201:placebo) ratio to receive either OP0201 or placebo within each dose cohort.

Populations for Analyses

Entered Analysis Set All subjects who sign the informed consent form.

Safety Analysis Set All subjects who receive any study treatment (OP0201 or placebo). Subjects will be analyzed according to the treatment they received.

Pharmacokinetic (PK) Analysis Set All subjects in Cohort B who received at least 1 dose of OP0201 and have 1 quantifiable serum DPPC or CP concentration collected post-dose without important protocol deviations/violations or events thought to significantly affect the pharmacokinetics.

Statistical Analyses

The Statistical Analysis Plan (SAP) will be developed and finalized before database lock and will describe the subject analysis sets to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

All analyses (with the exception of PK parameter analysis), summaries, and listings will be performed using SAS software (Version 9.4 or higher).

Pharmacokinetic Analyses

Pharmacokinetic analyses will be described in the SAP that will be finalized before database lock. All PK analyses will be performed on the PK Analysis Set.

Observed and baseline adjusted serum DPPC concentrations will be summarized by time point. Observed and baseline adjusted serum CP concentrations (if available) will be summarized by time point

Serum DPPC PK parameters (if any) will be summarized. Serum CP PK parameters (if available) will be summarized.

Safety Analyses

All safety analyses will be performed on the Safety Analysis Set.

Treatment-emergent adverse events (TEAEs) are defined as AEs that first occurred or worsened in severity after administration of study treatment.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). For each study treatment, numbers of TEAEs and incidence rates will be tabulated by preferred term and system organ class.

Treatment-emergent AEs by maximum severity, TEAEs by relationship to study treatment, serious adverse events (SAE), TEAEs leading to death, and TEAEs leading to discontinuation of study treatment will be tabulated for each study treatment.

All laboratory test results, vital signs measurements, and ECG results will be summarized for each treatment group using descriptive statistics at each visit for raw numbers and change from baseline.

Test results from otoscopy, tympanometry, nasal and epipharynx endoscopy, UPSIT olfactory test, and audiology pure tone hearing test will be listed and summarized as appropriate.

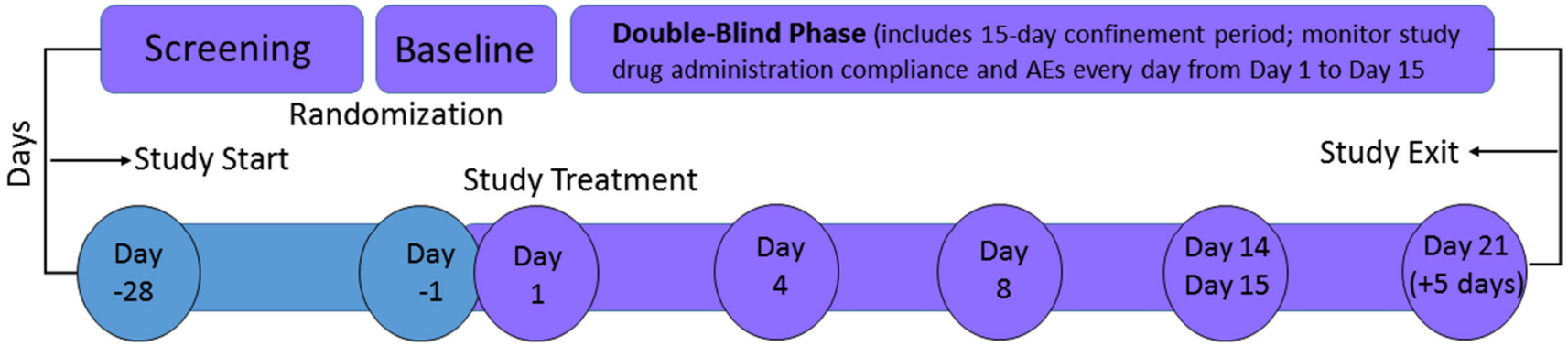
Safety Review Committee

After all subjects in Cohort A have completed the Day 14 visit, a SRC will review all the blinded safety data to determine whether the doses administered are safe and well tolerated and to make a recommendation to escalate to the next cohort (Cohort B) or not.

Data Monitoring Committee: Yes

1.2 Schema

Figure 1 Study Schema



1.3 Schedule of Activities

Table 3 Schedule of Activities

Study Period (with Visit Windows)	Screening Period	Confinement Period[a] (In-Clinic)						Study Exit	
	Days -28 to -1 (Screening) In-Clinic	Day -1 (Admission)	Day 1 (Enrollment)	Day 4	Day 8	Day 14	Day 15	Day 21 (+5 Day) In-Clinic	EaT[b]
Informed Consent/Authorization	X								
Demographics	X								
Inclusion/Exclusion	X	X							
Pregnancy Test (Urine)[c]	X	X						X	X
Follicle Stimulating Hormone Test[d]	X								
Urine Drug Screen[e]	X	X							
Viral Serology[f]	X								
Medical, Surgical, Ear History	X								
Physical Examination[g]	X							X	X
Vital Signs[h]	X	X	X	X	X	X	X	X	X
12-Lead ECG[i]	X		X			X		X	X
Ear History	X								
Otoscopy[j]	X	X		X	X	X			
Tympanometry	X	X		X	X	X		X[o]	X[p]
Nasal and Epipharynx Endoscopy[k]	X	X			X	X			
Clinical Laboratory Tests[l]	X					X			X
UPSIT Olfactory Test[m]	X				X	X		X[n]	X
Audiology Pure Tone Hearing Test	X					X		X[o]	X[p]

Study Period (with Visit Windows)	**Screening Period**	**Confinement Period[a] (In-Clinic)**						**Study Exit**	
	Days -28 to -1 (Screening) In-Clinic	**Day -1 (Admission)**	**Day 1 (Enrollment)**	**Day 4**	**Day 8**	**Day 14**	**Day 15**	**Day 21 (+5 Day) In-Clinic**	**EaT[b]**
Admission		X							
Train Participant on Study Product Administration[q]		X	X						
Randomization		X							
Study Treatment[r]			X	X	X	X			
Concomitant Medications	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X
PK Sample Collection			X[r]			X[s]			
Device Experience Questionnaire – Subject[t]							X		X[u]
Device Experience Questionnaire – Study Center Staff Administering the Treatment[t]							X		X[u]

EaT = Early termination; ECG = Electrocardiogram; IP = Investigational Product; PK = pharmacokinetic; UPSIT = University of Pennsylvania Smell Inventory Test.

a Eligible healthy volunteers will be admitted to the study center on Day -1 and be randomized on Day -1. Subjects will remain resident at the study center until after all the Day 15 assessments have been completed.

b EaT: To be performed if the subject was treated but early discontinue from the study.

c For all females, urine pregnancy test must be negative to meet study enrollment criteria.

d To be performed on postmenopausal women at Screening to confirm menopausal status (see Appendix 6).

e Specific tests to be conducted are listed in Table 8, Appendix 3.

f To be performed at Screening only (see Table 8, Appendix 3).

g 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 at the Screening visit.

h Vital signs include blood pressure, respiratory rate, pulse rate, and body temperature.

i A single 12-lead ECG will be collected for screening purposes. For subjects in Cohort A, a single 12-lead ECG will be collected on Day 1 prior to the first dose and on the study exit visit (Day 21). For subjects in Cohort B, triplicate ECGs will be collected on Day 1: 30 and 60 minutes prior to the first dose, Day 14: 30 minutes prior to the last dose and 5, 20, 35, 50 minutes following the last dose on Day 14. In cases where ECG and PK sampling overlap, ECGs should be collected first, to ensure that PK samples are collected at the scheduled time point.

j Otoscopy involves ear endoscopy, taking a picture of the tympanic membrane and assessing if there are any abnormal findings.

k Nasal endoscopy will include a measurement of nasal cavity length (in mm) at the Screening visit only. For all other scheduled assessments, the nasal and epipharynx endoscopy assessment should be done prior to the first dose of the day.

l Includes hematology, clinical chemistry, coagulation and urinalysis (see Table 8, Appendix 3).

m At Screening, a total UPSIT score of ≥35 (for females) and a total UPSIT score of ≥34 (for males) is required to meet study enrollment criteria.

n To be collected at the Day 21 study exit visit only if abnormal at the Day 14 visit. Abnormal is defined as UPSIT score <35 (for females) and <34 (for males) (see Section 8.2.8).

o Audiology pure tone hearing test if abnormal in either ear at Day 14 visit. Abnormal defined as >20 dB worsening from baseline at Day 14 visit (Rosenfeld et al, 2004). Typanometry is performed along with the Audiology pure tone hearing test so if the subject requires a hearing test at this visit, tympanometry will also be performed.

p Audiology pure tone hearing test and tympanometry not required if subjects drops out before Day 14, but if they drop out after Day 14 and had an abnormal test result at Day 14 visit, then every effort should be made to have the patient complete these assessments at the EaT visit.

q Study subjects need to be trained on study treatment administration on Day -1 or on Day 1, prior to the first dose.

r Study treatment dosing recommendation: the first dose at 9:00 am, second dose at 16:00 pm and the third dose at 23:00 pm.

s PK sample timing Cohort B only: Day 1: 30 and 60 minutes prior to the first dose, Day 14: 30 minutes prior to the last dose and 5, 20, 35, 50 minutes following the last dose on Day 14. The 5-minute PK sample should be collected within ±2 minutes of the scheduled time and the 20-minute PK sample should be collected within +5 minutes of the scheduled time. All other PK samples should be collected within ±5 minutes of the scheduled time.

t To be completed before discharge from the study center on the morning of Day 15 (See Appendix 7 for an example).

u This assessment is needed only if subjects discontinue the study prior to completing the questionnaire on Day 15.

2.0 INTRODUCTION

2.1 Study Rationale

OP0201 metered dose inhaler (MDI) (referred herein simply as OP0201) is a novel, intranasal drug-device surfactant product being developed by Novus Therapeutics, Inc., for the treatment and prevention of otitis media (OM). To date, there is no drug product that has been approved to treat or prevent OM.

OP0201 is comprised of a 20:1 fixed combination of dipalmitoylphosphatidylcholine (DPPC; a phospholipid surfactant) and cholesteryl palmitate (CP; a neutral phospholipid spreading agent) suspended in propellant (HFA 134a). There are no other ingredients (ie, no excipients, no fillers) in the formulation other than the active ingredients and the propellant. None of these ingredients contain any animal or human derivatives. Both DPPC and CP are highly endogenous surfactants in humans, including the Eustachian tube (ET), nasopharynx, and respiratory system.

The drug product will be delivered as a local treatment through each nostril using a metered dose inhalation device. It will be sprayed directly backwards into each nostril by way of the anterior nostrils (not upwards towards the osteomeatal opening to the maxillary and ethmoid sinuses as other common intranasal products) towards the lateral wall of the nasal cavity so that the usual nasal mucociliary clearance pathway can facilitate delivery of the drug to the ET.

This Phase 1 safety study is designed to evaluate the safety and tolerability of daily intranasal OP0201 use for 14 consecutive days in 30 healthy adults. The study will be randomized, double-blind, placebo-controlled, parallel-group, and includes a dose-escalation cohort design with evaluation of 30 mg per day (Cohort A) and 60 mg per day (Cohort B) of OP0201.

For more information about OP0201, please refer to the most recent Investigator's Brochure (IB).

2.2 Background

The ET is a compliant liquid-lined tube between the middle ear (ME) and the nasopharynx. The ET serves 3 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 (Chandrasekhar and Mautone, 2004; Siebert and Danner 2006). In children, ET dysfunction resulting in inflammation of the ME, referred to as OM, is often triggered by a viral infection of the upper respiratory system (Chonmaitree et al, 2008; Thomas et al, 2014; Schilder et al, 2016). Furthermore, the ET of infants and young children are short, floppy, horizontal and function poorly, which makes ME fluid flow more challenging (Rosa Olivares et al, 2015; Rovers et al, 2004). A viral infection increases mucus production as well as alters the properties of mucus in the nasopharyngeal region, resulting in diminished normal mucociliary clearance by mucosal cells of the ET and nasopharynx and creating inflammation in the area. This causes dysfunction

of the ET leading to negative ME. The presence of viruses and/or bacteria in the ME results in inflammation and build-up of mucus or liquid behind the tympanic membrane (ie, OM). Common forms of OM are acute otitis media (AOM) and otitis media with effusion (OME). Otitis media with effusion is a build-up of mucus/liquid behind the tympanic membrane without symptoms of an ear infection. Often, there are no symptoms of OME; or there are subtle findings (eg, ear rubbing, clumsiness, conductive hearing loss, and disturbed sleep). In contrast, AOM often occurs when bacteria or viruses get trapped in the ME and cause fever, ear pain and active (acute) inflammation. Both AOM and OME are the result of a dysfunctional ET.

Both DPPC and CP are established endogenous components in human nasal passages and ET and are present on the liquid surface of the nasal passage and ET mucosal lining, and the entire respiratory system. Among phospholipid compounds, DPPC is the most hydrophobic and surface active (Notter, 2000; Taeusch et al., 2002). The hydrophobic nature of DPPC causes it to spread very slowly over an aqueous surface. However, in the presence of a spreading agent (ie, CP), phospholipids easily "spread" along a surface pressure gradient of mucosal lined lumens (Colacicco and Basu, 1977; Notter, 2000). Together these 2 active ingredients effectively absorb to the mucosal air-liquid interface and reduce the interfacial surface tension of the ET, which reduces passive opening pressure required to open the ET. In other words, DPPC + CP promotes 'de-sticking' and restoration of physiologic activity of the ET. Thus, the 3 major ET physiologic functions (clearance of fluid from the ME to nasopharynx, ventilation of the ME to maintain ambient air pressure, and protection of the ME) are restored. In turn, it is hypothesized that acute symptoms of OM will be relieved by providing a physiologic opening to the closed ME space, and, with repeat usage, future OM episodes will be prevented. The surfactant is delivered locally to the nasopharyngeal tissue via the nostrils to reduce the local surface tension and pressure that is preventing the ET from functioning normally. By restoring ET functionality, OPO201 facilitates normal ventilation of the ME, which in turn corrects the negative ME pressure that causes otalgia (ear pain) in patients with AOM. Restoring ET function facilitates ME drainage, which in turn may resolve hearing difficulties in patients with OME. In patients at risk of developing OM, delivering the surfactant to the nasopharyngeal tissue via the nostrils may prevent ET dysfunction and in turn prevent AOM and/or OME.

2.3 Benefit/Risk Assessment

As this study is being conducted in healthy human subjects, a health benefit to the subject is not expected.

OP0201 has not yet been evaluated in humans in controlled clinical studies. Thus, information regarding expected adverse events (AEs) from controlled clinical studies is unknown. However, a limited amount of data from 9 human cases exposed to 1 or more doses of other formulations of the investigational drug product (200:1 and 16:1 ratio) is available (see Section 5.2.1 of the IB for further information).

Surfactants, such as DPPC and CP, are components in several pharmaceutical products that have received approval by health authorities, including United States (US) Food and Drug Administration (FDA) and many European Union (EU) countries, (eg, Alveofact®, Curosurf®, Infasurf®, and 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 (eg, Proventil HFA® [albuterol], Aerospan® [flunisolide]) and intranasal inhalers (eg, QNASL™ [beclomethasone]) containing active pharmaceutical agents for the management of asthma and chronic obstructive pulmonary disease. The device used to deliver OP0201 to the nostrils is similar to devices utilized in the delivery of other approved inhaled metered dose products.

The Sponsor will immediately notify the Principal Investigator if any additional safety or toxicology information becomes available during the study.

3.0 OBJECTIVES AND ENDPOINTS

Table 4 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of OP0201 30 mg per day (given as 2 sprays per nostril 3 times a day [TID] for 14 consecutive days) and OP0201 60 mg per day (given as 4 sprays per nostril TID for 14 consecutive days) compared to 0 mg placebo (HFA-134a only) administered in a similar parallel-group fashion TID for 14 consecutive days in healthy adult volunteers.	• Adverse events, otoscopy, tympanometry, nasal and epipharynx endoscopy, University of Pennsylvania Smell Inventory Test (UPSIT) olfactory test, audiology pure tone hearing test, triplicate 12-lead electrocardiogram, physical examination, vital signs, and clinical laboratory tests.
Secondary/Exploratory	
• To evaluate if any systemic exposure of dipalmitoylphosphatidylcholine (DPPC) can be detected in serum at levels higher than endogenous DPPC levels in serum in up to 15 healthy adult volunteers who will be evaluated in the Cohort B dose (OP0210 60 mg per day) versus placebo. • To evaluate if any systemic exposure of cholesteryl palmitate (CP) can be detected in serum at levels higher than endogenous CP levels in serum in up to 15 healthy adult volunteers who will be evaluated in the Cohort B dose (OP0210 60 mg per day) versus placebo.	• Maximum baseline adjusted serum concentration (C_{max}) of DPPC on Day 14. • Time to maximum concentration (t_{max}) of DPPC on Day 14. • Maximum baseline adjusted serum concentration (C_{max}) of CP on Day 14. • Time to maximum concentration (t_{max}) of CP on Day 14. • Additional parameters may be calculated, if appropriate.

4.0 STUDY DESIGN

4.1 Overall Design

This is a randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study in healthy volunteers to evaluate the safety and tolerability of OP0201 administered intranasally 3 times a day (TID) using a MDI compared to a matching placebo (HFA-134a minus active ingredients) administered intranasally TID using a MDI for 14 consecutive days (see Figure 1 for the study schematic). The plan is to enroll up to 30 subjects at 1 study center in the US. Two dose cohorts are planned (Cohort A and Cohort B; N=15 per cohort, see Cohort Dosing below, Table 5). Within each cohort, subjects will be randomized in a 4:1 ratio to receive either OP0201 or placebo. After all subjects in Cohort A have completed the Day 14 visit, a Safety Review Committee (SRC) will review all the blinded safety data to determine whether the doses administered are safe and well tolerated. The SRC will make a recommendation to escalate to the next cohort (Cohort B) or not.

Table 5 Dose Cohorts and Dose Regimen

Cohort	Dose Regimen	Cohort Dose Allocation		Total Subjects in Each Cohort	Total Subjects Per Dose Group
		Per Day Total Dose (mg)	Cumulative Total Dose (mg)		
A	**2 sprays per nostril TID x 14 days**	OP0201 (30 mg)[a] Placebo (0 mg)	OP0201 (420 mg)[a] Placebo (0 mg)	15	N=12 (OP0201) N=3 (Placebo)
B	**4 sprays per nostril TID x 14 days**	OP0201 (60 mg)[a] Placebo (0 mg)	OP0201 (840 mg)[a] Placebo (0 mg)	15	N=12 (OP0201) N=3 (Placebo)

a 2.5 mg per spray
N = number of subjects; TID = 3 times a day

Potential subjects will be screened for eligibility during a screening period (Days -28 to -1). Eligible healthy volunteers will be admitted to the study center on Day -1 and be randomized on Day -1. Subjects will remain resident at the study center until after all the Day 15 assessments have been completed (see Table 3 for the Schedule of Assessments [SoA]), with a study exit visit at Day 21.

Study treatment will be administered to the subjects by study center staff TID for 14 days while resident at the study center.

The estimated duration of participation in the study for each subject is up to 54 days: Screening (Days -28 to -1), treatment (Day 1), 16-day residency (Day -1 to Day 15 with safety assessments at Days 4, 8, 14 and 15), study exit (Day 21 [+5 days]). No efficacy assessments will be performed for this study.

4.2 Scientific Rationale for Study Design

This study is designed to evaluate the safety and tolerability of OP0201 versus placebo in healthy volunteers using a well-designed, scientific method for comparing treatments.

The study design is that of a well-controlled clinical study that includes elements necessary for a valid evaluation of safety and tolerability of repeat OP0201 doses for 14 days. The study is randomized, and treatment is masked for the Investigator, study center personnel, and the subjects to minimize bias. A parallel-group design eliminates possible confounding effects that are inherent in other study designs (eg, cross over).

The placebo (HFA-134a minus active ingredients) was selected to evaluate the comparative safety profile of OP0201 versus a non-drug containing control arm. HFA-134a was chosen as the placebo control since it is the propellant used in the OP0201 formulation. HFA-134a is used as a propellant in other marketed nasal products and has a good safety and tolerability profile in patients suffering from perennial allergic rhinitis (Weber et al, 2006).

The current study is designed to show that OP0201 is safe and well tolerated compared to the placebo (FDA Drug Study Designs - Information Sheet).

4.3 Justification for Dose

The US FDA has agreed that animal toxicology studies with OP0201 are not needed to proceed with evaluating this treatment in humans, since the active ingredients are present in other marketed products and the active ingredients are highly endogenous to the human respiratory tract. The composition of OP0201 is designed to maximize the lowering of the mucosal surface tension. Safety and proof of concept results from animal pharmacology studies are considered supportive of the formulation intended for human use. Across several nonclinical pharmacology studies, the beneficial effects of OP0201 were consistently better than those of no treatment and treatment with placebo alone (HFA-134a minus active ingredients) (see IB, Section 4, for more details). A multiple day dose of 20 mg twice daily was given to chinchillas for 10 days. The total exposure was calculated at 400 mg over 10 days; approximately 800 to 1100 mg/kg for a chinchilla weighing 0.35 to 0.5 kg (Chandrasekhar and Mautone, 2004). No AEs were observed in any of the animal studies.

The clinical safety and tolerability of OP0201 (DPPC:CP 20:1 [weight/weight] (w/w), manufactured by Novus Therapeutics, Inc.) for human use is further guided by a limited amount of data from 9 human cases exposed to 1 or more doses of other formulations of the investigational drug product (200:1 w/w and 16:1 w/w). A total of 8 cases were exposed to DPPC:CP 16:1 w/w in HFA-134a with each 0.1 mL spray delivering 2.5 mg active ingredient. One case exposed to DPPC:CP 200:1 w/w in HFA-134a, with each 0.1 mL spray delivering 5 mg of active ingredient (see IB, Section 5.2.1).

This Phase 1 safety study is designed to evaluate the safety and tolerability of OP0201 in 30 healthy volunteers. The dosing regimen for each of the 2 dose cohorts to be evaluated in this study are defined in Table 5. A TID dosing exceeds the anticipated once daily or maximum twice daily dosing interval that may be evaluated in patients in future studies. Because of the endogenous nature of the active ingredients, pharmacokinetic (PK) profiles cannot be obtained to guide on dose or dosing interval. Furthermore, the treatment is anticipated to have a local effect on the ET, and thus systemic plasma levels wouldn't necessarily have relevance. The maximum number of sprays of 4 per nostril to be evaluated in this Phase 1 study also exceed the anticipated practical maximum number of sprays of 2 per nostril that will be evaluated in patients in future studies. The dose, number of sprays, and dosing interval for this Phase 1 study were chosen because they are expected to exceed the total daily dose for dosing schema that will be tested in patients in future studies, and thus establish a high safety threshold.

The surfactant active ingredients of OP0201 are endogenous to human nasal and respiratory mucosal surfaces. Additionally, surfactants have been approved for topical administration via the trachea to infants with RDS with a long history of safety and tolerability at much higher exposure levels on a mg/kg basis than proposed herein (see IB, Section 4.1.5, Table 5). Thus, in this study daily repeat maximum doses of 20 mg TID for 14 days are to provide an estimated total exposure of 840 mg over 14 days; approximately 12 mg/kg for a human weighing 70 kg.

Rationale for Route of Administration

The target end organ for the study treatment is the ET, which exits the ME and resides in the nasopharynx. In adults, the nasal passage extends about 10 to 14 cm from the nostrils to the nasopharynx (Reznik, 1990). Access to the ET is via the nostril. Because of the physical location of the ET in the nasopharynx, the angle of the delivery of the investigational product (IP) in the nostril is in a horizontal direction towards the back of the nose, not vertical towards the sinus cavities (not 'up' into the nose) as for other nasal inhaled products. The IP is delivered using a MDI; thus there is no need for the user to sniff or breathe in a certain way for the IP to reach the ET.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed all phases of the study including the last visit.

The end of the study is defined as the date of the last visit of the last subject in the study.

4.5 Dose-Escalation Criteria

After all subjects in Cohort A have completed the Day 14 visit, a SRC will review all the blinded safety data to determine whether the doses administered are safe and well tolerated and to make a recommendation to escalate to the next cohort (Cohort B) or not.

4.6 Study Stopping Criteria

4.6.1 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. See Section 7.1 for general discontinuation procedures.

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

4.6.2 Criteria for Stopping Dose-Escalation

Dose-escalation will be stopped if any of the study stopping rules are met.

4.6.3 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.

5.0 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

Healthy volunteers are those who are otherwise healthy and who meet the eligibility criteria below.

5.1 Inclusion Criteria

Healthy volunteers are eligible to be included in the study only if all of the following criteria apply:

1. Subject has signed an informed consent form (ICF) before any study-specific procedures are performed.
2. Male or female healthy volunteers aged 18 to 50 years at the time of signed informed consent.
3. Able and willing to follow study instructions (including compliance with daily study treatment administration) and likely to complete all required study visits as assessed by the Investigator's judgement.
4. Body mass index of 18 to 30 kg/m^2 (inclusive) and a minimum body weight of 50 kg at Screening.
5. Female subjects must agree to use an acceptable method of contraception (see Appendix 6 for pregnancy considerations and contraceptive requirements).
6. Female subjects who are of childbearing potential must have a negative urine pregnancy test result at Screening and Day -1 prior to randomization.
7. Male subjects must agree to use contraception as detailed in Appendix 6 of this protocol during the treatment period.
8. Subjects must agree to refrain from immersing their head fully under water (eg, swimming, diving) from the time of signed informed consent until after the study exit visit (Day 21).
9. Physiologic tympanogram classified as Type A (normal) by the Investigator or designee.

5.2 Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

1. 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
   - ENT examination (ear history, otoscopy, tympanogram, nose and epipharynx endoscopy)
   - University of Pennsylvania Smell Inventory Test (UPSIT) olfactory test
   - Audiology pure tone hearing test
   - ECG
   - Vital signs measurements
   - Clinical laboratory tests
2. Presence of a clinically significant abnormal olfactory test finding at Screening defined as a total UPSIT score <35 (for females) and <34 (for males).
3. Current diagnosis of Sleep Apnea.
4. Clinically significant ear disorder/disease currently or within 6 weeks prior to Screening.
5. History of tympanostomy tubes in one or both ears within 1 year prior to Screening.
6. Upper respiratory tract infection or pharyngitis currently or within 6 weeks prior to Screening.
7. Allergy or sinus conditions (eg, sinusitis, non-specific nasal inflammation) currently or within 6 weeks prior to Screening.
8. Clinically relevant blockage of one or both nasal passages, in the Investigator's opinion.
9. Gastroesophageal reflux disease currently or within 6 weeks prior to Screening.
10. Smoker (eg, cigarettes, vapor) or tobacco or nicotine use within the last 48 weeks prior to Screening and positive urine cotinine test results at Screening or Day -1 prior to randomization.
11. Females with positive urine pregnancy results at Screening or Day -1 prior to randomization.
12. Females who are nursing or planning a pregnancy during the study.
13. Females of childbearing potential who are not willing to use an acceptable method of contraception during the study (see Appendix 6).

14. Symptomatic herpes zoster within 12 weeks prior to Screening.
15. Lymphoma, leukemia or any malignancy within the past 5 years except for basal cell or squamous epithelial carcinomas of the skin that have been resected with no evidence of metastatic disease for 3 years.
16. Breast cancer within the past 10 years.
17. Live vaccine within 30 days prior to Screening, or plans to receive such vaccines during the study.
18. Evidence of craniofacial anomalies (eg, cleft palate, Down's Syndrome).
19. Disorders with decrease mucociliary clearance or higher viscosity of the mucous (eg, cystic fibrosis, primary ciliary dyskinesia, Karfagener's syndrome).
20. Use of any medication (either topically or systemically) for a condition related to the ear or nose currently or within 12 weeks prior to Screening.
21. Use of medications with known vasoconstrictive properties (eg, local anesthetics, decongestants) currently or within 2 weeks prior to Screening.
22. Use of medications with known anti-cholinergic side effects (eg, antihistamines, antiemetics, muscle relaxants, antidepressants) currently or within 6 weeks prior to Screening.
23. Within 7 days prior to the first study treatment administration, use of any prescription drug (except oral contraceptive), nicotine-containing product (including nicotine patch), over-the-counter drug, herbal supplement, vitamin, or dietary supplement with the exception for occasional use of acetaminophen not exceeding 3 grams per day.
24. Regular alcohol consumption within 24 weeks prior to Screening defined as an average weekly intake of >21 units for males and >14 units for females; 1 unit is equivalent to 8 g of alcohol (a half pint [approximately 240 mL] of beer, 1 glass [approximately 125 mL] of wine or 1 measure [approximately 25 mL] of spirit).
25. History of drug abuse or positive drug screening test at Screening or Day -1 prior to randomization (see Table 8, Appendix 3).
26. Known allergy or sensitivity to the study treatment or any of its components.
27. Exposure to 3 or more new chemical entities within 12 months prior to Screening.
28. Investigator and study center personnel directly affiliated with this study and/or their immediate families (defined as spouse, significant-other, parent, child or sibling, whether adopted or biologic).

29. Persons employed by the Sponsor or Investigator.
30. Persons held in an institution by legal or official order.
31. Concurrent enrollment in an investigational drug or device study other than the research pertaining to OP0201, or participation in such a study within 30 days of Screening.
32. Subject has a condition or is in a situation which, in the Investigator's opinion, may put the subject at significant risk, may confound the study results, or may interfere significantly with the subject's participation in the study.

5.3 Lifestyle Considerations

Please see Section 6.5 for medications that are prohibited during this study.

Subjects must refrain from immersing their head fully under water (eg, swimming, diving) from the time of signed informed consent until after the final study exit visit (Day 21).

5.3.1 Meals and Dietary Restrictions

1. Subjects should abstain from consuming grapefruit, grapefruit juice, cranberry juice, and other cranberry containing products or any products made with Seville oranges (eg, orange marmalade) from 14 days before admission to the study center until after the final study exit visit (Day 21).

5.3.2 Caffeine and Alcohol

1. Subjects will abstain from caffeine or xanthine-containing products for at least 48 hours prior to admission to the study center and for the duration of the stay in the study center.
2. Subjects will abstain from alcohol from Screening until after the final study exit visit (Day 21).

5.3.3 Activity

1. All subjects must comply with the contraception requirements as noted in Appendix 6.
2. Subjects should refrain from strenuous exercise for 24 hours before Screening, before admission and for the duration of the study.
3. Subjects will be advised not to donate blood or plasma for at least 3 months after the last dose administration.

5.4 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomly assigned to study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography (eg, age, gender, race), reasons for screen failure (eg, note which eligibility criteria was not met), and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

6.0 STUDY TREATMENT

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a subject according to the study protocol.

The study treatment is to be used in accordance with the protocol for healthy volunteers who are under the direct supervision of a qualified Investigator.

6.1 Study Treatment(s) Administered

The following study treatments will be used in this study.

Study Treatment #1: OP0201

OP0201 is an intranasal aerosol, a dosage form recognized by the US Pharmacopeia, that is comprised of 2 active ingredients, DPPC and CP, in approximately 20:1 (w/w) ratio, suspended in an inactive ingredient, the synthetic chlorofluorocarbon-free propellant HFA 134a. All OP0201 ingredients are synthetic; none of these ingredients contain any animal or human derivatives. OP0201 is supplied in mechanical packing parts that includes a pressurized canister with a securely attached metering valve where the drug product is contained, an actuator into which the canister is seated and the valve connects with device body, and a tip to deliver the drug to the nostrils.

OP0201 is manufactured using Good Manufacturing Processes (GMP) with development phase appropriate compliance with global Health Authority guidelines (eg, US FDA Guidance for Industry: Nasal Spray and Inhalation Solution, Suspension and Spray Drug Products, July 2002) by [redacted]. The OP0201 canisters are filled to deliver 100 actuations (sprays) per canister. Each MDI spray of OP0201 delivers 0.1 mL of HFA-134a containing a total of 2.5 mg of the active pharmaceutical ingredients as dry powder suspended in the HFA-134a.

See the IB for further details regarding OP0201.

Study Treatment #2: Placebo

The placebo is comprised of the same synthetic chlorofluorocarbon-free propellant, HFA-134a, an inactive ingredient as used in the study treatment #1 described above. The placebo is manufactured using GMP with development phase appropriate compliance with global Health Authority guidelines (eg, US FDA Guidance for Industry: Nasal Spray and Inhalation Solution, Suspension and Spray Drug Products, July 2002) by [redacted]. The placebo canisters are filled to deliver 100 actuations (sprays) per canister. Each 0.1 mL MDI spray of placebo delivers 0.1 mL of HFA-134a.

For each dose, the subject will have administered to them by the study center staff, 2 (Cohort A) or 4 (Cohort B) consecutive sprays first to the left nostril and then to the right nostril for a total of 4 (Cohort A) or 8 (Cohort B) sprays (see Study Manual for Study Treatment Instructions for Use). The dose will be repeated 3 times a day for a total of 14 days (42 doses).

The study treatment will be administered every 7 hours: a morning dose at 9:00 am, an afternoon dose at 16:00 pm and an evening dose at 23:00 pm. The Investigator or qualified designee will record each dose by noting the time and number of total sprays administered successfully in a dosing log.

6.2 Preparation/Handling/Storage/Accountability

1. The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
2. Only subjects enrolled in the study may receive study treatment and only authorized study center staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized study center staff.
3. The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

The Investigator, a member of the study center staff, or a pharmacist must maintain an adequate record of the receipt and distribution of all study medication using the Drug Accountability Form. These forms must be available for inspection at any time.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Study Treatment Kit

Each study treatment kit will be labeled with a kit number and contain a predetermined number of units. For this study, a unit is defined as 1 canister of study treatment. Each kit will include the necessary amounts of units of study treatment; according to the study treatment cohort and dose level. Further details will be provided in the Laboratory Manual.

On Day 1 subjects will be assigned a unique number (randomization number) in ascending numerical order. The randomization number encodes the subject's assignment to 1 of the 2 study treatments of the study, according to the randomization schedule generated prior to the study by the Statistics Department at the Sponsor or designee. Each subject will be assigned blinded study treatment, labeled with his/her unique randomization number, that will be used throughout the study.

Within each dose cohort, subjects will be randomly assigned in a 4:1 ratio to receive OP0201 or placebo. The randomization scheme will be prepared by the Sponsor's designee.

6.3.2 Blinding

Study treatment will be provided in identically appearing canisters and kits to maintain masking of the study.

6.3.3 Procedures for Unblinding Study Treatment

A sealed envelope that contains the study treatment assignment for each subject will be provided to the Investigator. The sealed envelope will be retained by the Investigator (or representative) in a secured area. In case of an emergency, the Investigator has the sole responsibility for determining if unblinding of a subject's treatment assignment is warranted. Subject safety must always be the first consideration in making such a determination. If the Investigator decides that unblinding is warranted, the Investigator should make every effort to contact the Sponsor and Medical Monitor prior to unblinding a subject's treatment assignment unless this could delay emergency treatment of the subject. If a subject's treatment assignment is unblinded, the Sponsor and Medical Monitor must be notified within 24 hours after breaking the blind. Once the study is complete, all envelopes (sealed and opened) must be inventoried and returned to the Sponsor.

6.4 Study Treatment Compliance

The prescribed dosage, timing, and mode of administration may not be changed, except as defined in Section 6.6.

Only subjects enrolled in the study may receive study treatment and only authorized study center staff may administer the study treatment to the subject.

The Investigator or qualified designee will record each dose by noting the time and number of total sprays administered successfully in a dosing log.

6.4.1 Treatment Strategy for Emergencies

The clinical study center is a large clinical pharmacology Inpatient Unit that has 24-hour staffing and security coverage 7 days a week and is located within 5 miles of 3 local hospitals, one of which is a trauma center. Highly trained Paramedic/Emergency Medical Technicians (EMT) are available in the study center on a 24-hour basis and are trained to deal specifically with life-threatening AEs and medical emergencies. All EMTs are Advance Cardiovascular Life Support certified, and all study center staff are Cardiopulmonary Resuscitation certified. All equipment necessary for resuscitative care is available in the study center. The study center is equipped with 24/7 video surveillance/controlled access throughout the facility, strategically located panic buttons, 48-channel cardiac telemetry and continuous pulse oximetry and 2 emergency crash carts.

6.4.2 Warnings and Precautions

As this is the first administration of OP0201 to healthy human volunteers, all effects cannot be reliably predicted. The preclinical data suggest an acceptable safety profile. In addition, the active pharmaceutical ingredients in the study treatment are endogenous to humans. Facilities and staff for resuscitation and the treatment of other medical emergencies will be provided.

6.5 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment (within 7 days before the time of enrollment) or receives during the study must be recorded on the electronic case report form (eCRF) along with:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including dose and frequency.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Subjects must abstain from taking prescription or nonprescription drugs (including vitamins and dietary or herbal supplements) within 7 days or 5 half-lives (whichever is longer) before the start of study treatment until completion of the study exit visit (Day 21). In the case of medications (either topically or systemically) for a condition related to ear or nose, subjects must abstain from taking these within 12 weeks before the start of study treatment, or 2 weeks in the case of medications with known vasoconstrictive properties (eg, local anesthetics, decongestants), or 6 weeks in the case of medications with known anti-cholinergic side effects (eg, antihistamines, antiemetics, muscle relaxants, antidepressants). An exception is that women of childbearing potential (WOCBP) may take protocol defined acceptable oral contraceptives.

Acetaminophen, at doses of $\leq$ 3 grams/day, is permitted for use any time during the study. Other concomitant medication may be considered on a case-by-case basis by the Investigator in consultation with the Medical Monitor (if required).

6.6 Dose Modification

Dose modifications are not planned in this study.

6.7 Treatment After the End of the Study

The Sponsor will not provide any additional care to subjects after they leave the study.

7.0 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT DISCONTINUATION/WITHDRAWAL

If a clinically significant finding is identified, the Investigator or qualified designee will determine if the subject should continue with the study treatment and if any change in subject management is needed, and/or if they should remain in the study. Any new clinically relevant finding should be reported as an AE.

7.1 Discontinuation of Study Treatment

Subjects should not receive further study treatment if one or more of the following criteria are met. It is not necessary to exit the participant from the study as long as they do not receive further study treatment. The Investigator may use their discretion in deciding whether to discontinue the participant from the study. If the subject is discontinued from the study early, see SoA (Section 1.3) for data to be collected at the time of study discontinuation. The reason that the study treatment is stopped should be documented on the appropriate eCRF.

7.2 Subject Discontinuation/Withdrawal from the Study

Randomized subjects who meet any of the following criteria should not receive further study treatment and should be exited from the study. See SoA (Section 1.3) for data to be collected at the time of study exit.

- Investigator or Sponsor (or its designee) decides to discontinue subjects from the study for safety, behavioral, compliance, or administrative reasons.
- Investigator or Sponsor (or its designee) decides to discontinue subjects from the study who are inadvertently enrolled into the study despite significant deviation from protocol-specified inclusion/exclusion criteria.
- Subject indicates that they no longer want to participate in the study. If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the study center study records.

Notification of early subject withdrawal from the study and the reason for discontinuation will be clearly documented and noted in the eCRF. If the subject exits the study prior to the Day 21 study exit visit, all of the Day 21 final measurements should be performed and recorded on the appropriate eCRF.

Subjects who are discontinued from the study may be replaced based on the Sponsor's discretion.

7.3 Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The study center must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

8.0 STUDY ASSESSMENTS AND PROCEDURES

This section provides a high-level summary of the study assessments to be performed. For details as to when these assessments are performed see the SoA (Section 1.3)

- Study procedures and their timing are summarized in Section 1.3.
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor designee immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subjects' routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each subject over the duration of the study, including any extra assessments that may be required, will not exceed 450 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1 Efficacy Assessments

This is a Phase 1 safety study, there are no efficacy assessments.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.2.1 Physical Examinations

- A complete physical examination will be performed to assess any physical abnormalities and will include at a minimum, assessments of the following body systems: general appearance, overall status of the skin, head, neck, trunk, eyes, heart and lungs (eg, breathing sounds), abdomen, extremities, lymph nodes.
- Height and weight will also be measured and recorded at the Screening visit.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2 Vital Signs

Vital signs are to be taken before blood collection for laboratory tests.

- Systolic and diastolic blood pressure (mmHg): to be assessed after a participant has been at rest (seated) for at least 5 minutes in a quiet setting without distractions (eg, television, cell phones) with a completely automated device. Manual techniques will be used only if an automated device is not available. A total of 3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute. The average of the 3 blood pressure readings will be recorded on the eCRF.
- Pulse rate (beats/minute): to be assessed after a subject has been at rest (seated) for at least 5 minutes in a quiet setting without distractions (eg, television, cell phones) with a completely automated device. Manual techniques will be used only if an automated device is not available. A total of 1 pulse reading should be recorded.
- Respiration rate (per minute) will be measured by counting at least 30 seconds and multiplying accordingly. Respiration rate may be taken concurrently with all ECGs.
- Oral temperature (as Fahrenheit degrees) will be assessed and recorded.

8.2.3 Electrocardiograms

- A single 12-lead ECG will be performed at the Screening visit to ensure that a study subject does not have, in the Investigator's clinical judgement, a clinically significant condition or a condition that may pose an unacceptable safety risk for participation in the study.
- For subjects in Cohort A, a single 12-lead ECG will be collected on Day 1 prior to the first dose and on the study exit visit (Day 21).
- For subjects in Cohort B, triplicate 12-lead ECGs will be collected at subsequent visits as outlined in the SoA (see Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.
- In cases where ECG and PK sampling overlap, ECGs should be collected first, to ensure that PK samples are collected at the scheduled time point.
- At each time point at which triplicate ECGs are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.
- ECGs will be assessed by a central ECG reading center. A cardiologist will review screening, pre- and post-treatment ECGs and discuss any abnormal findings with the Investigator, as appropriate.

8.2.4 Clinical Safety Laboratory Assessments

- See Appendix 3 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.
    - If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
    - All protocol-required laboratory assessments, as defined in Appendix 3, must be conducted in accordance with the Laboratory Manual and the SoA.
    - If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the Investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Otoscopy

Otoscopy will be performed to assess the appearance of the tympanic membrane (normal or abnormal). If abnormal, then it will be rated for the following variables:

- Contour (retracted, full, bulging, perforated).
- Color (partly red or completely red).
- Color of fluid behind the tympanic membrane (yellow, dark red, bright red, green, or clear).
- Translucency (semi-opaque, opaque).

8.2.6 Tympanometry

Tympanometry will be performed to assess tympanic membrane mobility, ET function and ME function by measuring the amount of sound energy reflected back when a small probe is placed in the ear canal (Rosenfeld et al, 2016). The output of the right ear and left ear tympanometry will be a tympanogram tracing classified as Type A (normal), Type B (abnormal) or Type C (abnormal) (Jerger, 1970).

8.2.7 Nasal and Epipharynx Endoscopy

Nasal and epipharynx endoscopy will be assessed for normal or abnormal appearance. Under abnormal, the following criteria should be assessed:

- Swelling
- Redness
- Bleeding
- Rhinorrheoea (nose only)
- Other

At the Screening visit, as a part of the nasal endoscopy, the length of the nasal cavity (in mm) will be recorded separately for the left and right side. The length of the nasal cavity is defined as the distance from the tip of the nose to the entry point of the ET.

For all other scheduled assessments, the nasal and epipharynx endoscopy assessment should be done prior to the first dose of the day.

8.2.8 Olfactory Test

An olfactory test, UPSIT, will be performed at the Screening (Day -28 to Day -1) visit to ensure that a subject does not have a clinically significant condition or a condition that may pose an unacceptable safety risk for participation in the study. At the Day 8 and Day 14 visits, an olfactory test will be performed to assess for abnormal olfactory function after the dosing period. If abnormal findings are present at the Day 14 visit, then an additional olfactory test will be performed at the Day 21 study exit visit.

The UPSIT test consists of 4 booklets containing 10 odorants apiece, 1 odorant per page. The stimuli are embedded in 1 µm to 50 µm diameter microencapsulated crystals located on brown strips at the bottom of each page. Above each odorant strip is a multiple-choice question with 4 alternative responses for each item. For example, 1 of the items reads, “This odor smells most like: a) chocolate; b) banana; c) onion; or d) fruit punch.” The response “banana” is correct in this case. The subject is required to answer 1 of the 4 alternatives, even if no smell is perceived (ie, the test is forced-choice). This helps to encourage the subject to sample each odorant and provides a basis for the detection of malingering. To ensure uniformity in test administration, the test booklets should be administered in chronologic order, eg, booklet 1, booklet 2, etc. (Doty 1984). A total UPSIT score of <35 is abnormal for females and a total score <34 is abnormal for males. In this study we intend to determine if there is any change from ‘normal’ (at baseline) to ‘abnormal’ post-treatment. If such a change occurs it will be captured as an AE. The severity of the AE will be assessed as per the resulting ‘olfactory diagnosis’ in the following table:

Test Score	Olfactory Diagnosis
00-05	Probable Malingering
06-18	Total Anosmia
19-25	Severe Microsmia
26-29	Moderate Microsmia (males)
26-30	Moderate Microsmia (females)
30-33	Mild Microsmia (males)
31-34	Mild Microsmia (females)
34-40	Normosmia (males)
35-40	Normosmia (females)

8.2.9 Audiology Pure Tone Hearing Test

An audiology pure tone hearing test will be performed at the Screening (Day -28 to Day -1) visit to ensure that a subject does not have clinically significant abnormal hearing. Average pure tone hearing loss at 4 frequencies (500, 1000, 2000 and 4000 Hz) ranges from normal hearing to moderate hearing loss (0-55 dB) will be tested. The 50th percentile is about 25 dB hearing level (HL). Average pure tone audiometry will be performed as per the audiology testing template in Appendix 10. The hearing test should be performed in a quiet environment, preferably a separate closed or sound-proofed area set aside specifically for that purpose. Comprehensive audiologic evaluation is recommended for anyone who fails the pure tone hearing test. Audiologic assessment includes evaluating air-conduction and bone-conduction thresholds for pure tones, speech detection or speech recognition thresholds, and measurements of speech understanding, if possible (Rosenfeld et al, 2004).

8.3 Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

Adverse events will be reported by the subject.

The Investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment or the study (see Section 4.6 and 7.0).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the time first exposure to the study treatment until the study exit.

All AEs will be collected from the time first exposure to the study treatment until the study exit.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded as pre-treatment-emergent AEs in the AE section of the eCRF.

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Appendix 4. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

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.

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

8.3.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

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 (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 4.

8.3.4 Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and Investigators.

- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

For purposes of this study, a woman will be considered of childbearing potential unless documentation indicates she is permanently sterilized (ie, hysterectomy, bilateral salpingectomy or bilateral oophorectomy) 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 are permanently sterilized or are naturally postmenopausal will not be required to use contraceptives during the study.

The Investigator will review the contraception requirements for the study with each WOCBP who is considering being a subject.

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Appendix 6.

Details of all pregnancies in female subjects will be collected after the start of study treatment and until the study exit. If a pregnancy is reported, the Investigator should inform the Sponsor designee within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 6.

The Investigator will (1) notify the subject's physician that the subject was enrolled in the study and treated with an investigational drug OP0201 or placebo, and (2) follow the progress of the pregnancy to term. The Investigator must document the outcome of the pregnancy and provide a copy of the documentation to the Sponsor.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.3.6 Adverse Events of Special Interest

Not applicable.

8.3.7 Medical Device Incidents (Including Malfunctions)

Instructions for documenting Medical Device Incidents are provided in Appendix 9.

Medical devices are being provided for use in this study for delivering the study treatment via MDI. In order to fulfill regulatory reporting obligations worldwide, the Investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the study with such devices.

The definition of a Medical Device Incident can be found in Appendix 9.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 4 of the protocol.

8.3.7.1 Time Period for Detecting Medical Device Incidents

- Medical Device Incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.
- If the Investigator learns of any incident at any time after a participant has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the Investigator will promptly notify the Sponsor.

The method of documenting Medical Device Incidents is provided in Appendix 9.

8.3.7.2 Follow-up of Medical Device Incidents

- All Medical Device Incidents involving an AE will be followed and reported in the same manner as other AEs (see Appendix 4). This applies to all subjects, including those who discontinue study treatment.
- The Investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the Investigator.

8.3.7.3 Prompt Reporting of Medical Device Incidents to Sponsor

- Medical Device Incidents will be reported to the Sponsor within 24 hours after the Investigator determines that the event meets the protocol definition of a Medical Device Incident.
- The Medical Device Incident Report Form will be sent to the Sponsor by email. If email is unavailable, then standard post should be utilized.
- The same individual will be the contact for the receipt of medical device reports and SAE.

8.3.7.4 Regulatory Reporting Requirements for Medical Device Incidents

- The Investigator will promptly report all incidents occurring with any medical device provided for use in the study in order for the Sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The Investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

8.4 Treatment of Overdose

It is unknown what dose of study treatment within a 24-hour time period would be considered an overdose. Both active ingredients in the study treatment are endogenous to the human respiratory system in very large quantities compared with the maximum study treatment dose of 40 mg per day of surfactant. Even if a subject were to administer an entire canister of the study treatment into the nostrils in 1 day, the total exposure to the active pharmaceutical ingredient would be 250 mg (approximately 3.6 mg/kg for a 70 kg adult), which is far less than exposures on a mg/kg basis for approved surfactant products that are usually given over 1 to 2 days (see Table 5 of the IB).

HFA-134a is not endogenous to the human respiratory system. As noted in Section 4.3.2 of the IB, misuse or intentional inhalation abuse may cause death without warning symptoms, due to cardiac effects. Other symptoms potentially related to misuse or inhalation abuse are: anesthetic effects, light-headedness, dizziness, confusion, incoordination, drowsiness, or unconsciousness, irregular heartbeat with a strange sensation in the chest, heart thumping, apprehension, feeling of fainting, dizziness or weakness. Vapors are heavier than air and can cause suffocation by reducing oxygen available for breathing. However, the maximum amount of 10 mL of HFA-134a per study treatment canister would not be sufficient exposure to be considered an overdose. If symptoms described above were experienced with a suspected HFA-134a overdose, the suggested treatment would be to deliver oxygen and other necessary supportive care per the Investigator's judgement.

In the event of an overdose, the Investigator should:

- Contact the Medical Monitor immediately.
- Closely monitor the participant for any AE/SAE and laboratory abnormalities for at least 2 days.
- Document the quantity of the excess dose as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the subject.

8.5 Pharmacokinetics

Planned time points for all PK assessments are provided in the SoA (Section 1.3).

8.5.1 Collection of Samples

On Days 1 and 14, blood samples will be collected for the determination of DPPC and CP concentrations only for subjects in Cohort B. The SoA (Section 1.3) presents the schedule for blood collection. Time 0 is defined as the time of the study treatment administration.

The timing of scheduled samples may be adjusted according to clinical needs or needs for PK data. The actual collection times must be recorded. The time and date of study treatment administration will also be recorded. Instructions for the collection and handling of biological samples will be provided by the Sponsor or designee. Blood samples will be collected via a venous cannula or direct venipuncture. Samples will be collected, labeled, stored, and shipped as detailed in the Laboratory Manual. The total volume of blood taken for PK and pharmacodynamic sampling during the study will not exceed 450 mL. For specific sampling volumes, please refer to the Laboratory Manual.

The 5-minute PK sample should be collected within ±2 minutes of the scheduled time and the 20-minute PK sample should be collected within +5 minutes of the scheduled time. All other PK samples should be collected within ±5 minutes of the scheduled time.

8.5.2 Determination of Drug Concentration

Samples for the determination of DPPC and CP in serum will be analyzed on behalf of the Sponsor by [redacted] using appropriate validated bioanalytical methods. Full details of the bioanalytical methods will be described in a separate Bioanalytical Report.

All samples still within the known stability of DPPC and CP at the time of receipt by the bioanalytical laboratory will be analyzed.

Baseline samples collected from subjects who received placebo will be analyzed to establish the endogenous DPPC and CP concentrations. Samples collected after dosing from subjects who received placebo will not be analyzed.

Samples collected for analyses of DPPC and CP concentration may also be used to evaluate safety aspects related to concerns arising during or after the study.

8.5.3 Calculation of Derivation of Pharmacokinetic Variables

Pharmacokinetic parameters will be derived using noncompartmental methods with Phoenix® WinNonlin® Version 6.4 or higher (Certata, L.P. Princeton, New Jersey, US) and/or SAS® Version 9.2 or higher (SAS Institute, Inc., Cary, North Carolina, US). Actual elapsed time from

dosing and baseline adjusted DPPC and CP concentrations will be used for the final serum PK parameter calculations.

Serum Pharmacokinetic Parameters

The PK parameters in Table 6 will be determined for serum DPPC and CP on Day 14, when possible.

Table 6 Serum Blood Pharmacokinetic Parameters

Pharmacokinetic Parameter	Definition
C_{max}	Maximum concentration, obtained directly from the baseline adjusted concentration versus time data.
t_{max}	Time to C_{max}.

Additional serum parameters may be calculated if deemed appropriate.

Drug concentration information that may unblind the study will not be reported to the study center or study personnel until the study has been unblinded.

8.6 Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

8.7 Genetics

Genetics are not evaluated in this study.

8.8 Biomarkers

Biomarkers are not evaluated in this study.

8.9 Health Economics

Health Economics parameters are not evaluated in this study.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

Not applicable.

9.2 Sample Size Determination

The sample size is not based on statistical considerations, but is typical for studies of this nature, and is considered adequate to characterize the distribution of the planned endpoints.

There will be a total of 30 subjects; 2 dose cohorts of 15 subjects each. Subjects will be randomized in 4:1 (OP0201:placebo) ratio to receive either OP0201 or placebo within each dose cohort.

9.3 Populations for Analyses

For purposes of analysis, the analysis sets in Table 7 are defined.

Table 7 Analysis Sets

Analysis Set	Description
Entered Analysis Set	All subjects who sign the informed consent form.
Safety Analysis Set	All subjects who receive any study treatment (OP0201 or placebo). Subjects will be analyzed according to the treatment they received.
Pharmacokinetic Analysis Set	All subjects in Cohort B who received at least 1 dose of OP0201 and have 1 quantifiable serum DPPC or CP concentration collected post-dose without important protocol deviations/violations or events thought to significantly affect the pharmacokinetics.

CP = cholesteryl palmitate; DPPC = dipalmitoylphosphatidylcholine

9.4 Statistical Analyses

The Statistical Analysis Plan (SAP) will be developed and finalized before database lock and will describe the subject analysis sets to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

All analyses (with the exception of PK parameter analysis), summaries, and listings will be performed using SAS software (Version 9.4 or higher).

Descriptive statistics, including the following, will be used as applicable to summarize the study data:

- Continuous variables: sample size (n), mean, standard deviation (SD), median, minimum (min), and maximum (max).
- Categorical variables: frequencies and percentages.

Individual subject data will be presented in listings.

9.4.1 Efficacy Analyses

Not applicable.

9.4.2 Pharmacokinetic Analyses

Pharmacokinetic analyses will be described in the SAP that will be finalized before database lock. All PK analyses will be performed on the PK Analysis Set.

Observed and baseline adjusted serum DPPC concentrations will be summarized by time point. Observed and baseline adjusted serum CP concentrations (if available) will be summarized by time point.

Serum DPPC PK parameters (if any) will be summarized. Serum CP PK parameters (if available) will be summarized.

9.4.3 Safety Analyses

All safety analyses will be performed on the Safety Analysis Set.

Treatment-emergent adverse events (TEAEs) are defined as AEs that first occurred or worsened in severity after administration of study treatment.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). For each study treatment, numbers of TEAEs and incidence rates will be tabulated by preferred term and system organ class.

Treatment-emergent AEs by maximum severity, TEAEs by relationship to study treatment, SAEs, TEAEs leading to death, and TEAEs leading to discontinuation of study treatment will be tabulated for each study treatment.

All laboratory test results, vital signs measurements, and ECG results will be summarized for each treatment group using descriptive statistics at each visit for raw numbers and change from baseline.

Test results from otoscopy, tympanometry, nasal and epipharynx endoscopy, UPSIT olfactory test, and audiology pure tone hearing test will be listed and summarized as appropriate.

Additional details will be provided in SAP.

9.4.4 Missing Data

Missing data, if any, will be described in the SAP.

9.5 Interim Analyses

No interim analysis is planned.

9.6 Safety Review Committee

After all subjects in Cohort A have completed the Day 14 visit, a SRC will review all the blinded safety data to determine whether the doses administered are safe and well tolerated and to make a recommendation to escalate to the next cohort (Cohort B) or not.

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11.0 APPENDICES

Appendix 1 Abbreviations

Term/Abbreviation	Definition
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOM	Acute Otitis Media
C_{max}	Maximum concentration
CRF	Case Report Form
CP	Cholesteryl palmitate
DPPC	Dipalmitoylphosphatidylcholine
ECG	Electrocardiogram
EaT	Early termination
ET	Eustachian tube
eCRF	Electronic case report form
EU	European Union
FSH	Follicle stimulating hormone
FDA	Food and drug administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Processes
HL	Hearing level
HRT	Hormonal replacement therapy
IB	Investigator's Brochure
ICH	International Council for Harmonisation
ICF	Informed consent form
INR	International normalized ratio
IP	Investigational product
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MDI	Metered dose inhaler
ME	Middle ear
PK	Pharmacokinetics
OM	Otitis Media
OME	Otitis Media with Effusion
RDS	Respiratory distress syndrome

SAE	Serious adverse event
SAP	Statistical Analysis Plan
SoA	Schedule of Assessments
SRC	Safety Review Committee
t_{max}	Time to C_{max}
TEAE	Treatment-emergent adverse event
TID	Three times a day
ULN	Upper limit of normal
US	United States
UPSIT	University of Pennsylvania Smell Inventory Test
WOCBP	Woman of childbearing potential

Appendix 2 Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines.
  - Applicable ICH Good Clinical Practice (GCP) Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each Investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or representative (Appendix 12). The study will not start at any study center at which the Investigator has not signed the protocol.

Adequate Resources

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study-related duties and functions conducted at the study center.

If the Investigator/institution retains the services of any individual or party to perform study-related duties and functions, the Investigator/institution should ensure this individual or party is qualified to perform those study-related duties and functions and should implement procedures to ensure the integrity of the study-related duties and functions performed and any data generated.

Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Insurance

The Sponsor has obtained liability insurance, which covers this study as required by local law and/or national regulations and/or ICH guidelines, whichever is applicable. The terms of the insurance will be kept in the study files.

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

- The Sponsor or its representative will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician, or any other third party, unless required to do so by law.
- Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, the Sponsor or representative physician or an Investigator might know a subject's identity and also have access to his or her genetic data. Also, regulatory authorities may require access to the relevant files.

Administrative Structure

The safety committee will include, at a minimum, the Sponsor's responsible physician (or designated Medical Monitor) and the Principal Investigator. The Principal Investigator and the Sponsor, when appropriate, will invite other specialist individuals to participate in the review, eg, PK scientists, statisticians, clinical specialists etc.

Medical Monitor

[redacted]

Dissemination of Clinical Study Data

The results of the study should be reported within 1 year from the end of the clinical study. The study will be registered on clinicaltrials.gov and appropriate reporting to ensure compliance with clinicaltrials.gov will be met.

Data Quality Assurance

- All subject data relating to the study will be recorded on printed or eCRFs unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the case report form (CRF).
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

The Investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (eg, via an audit trail).

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's study center.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Study Center Closure

The Sponsor designee reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed.

The Investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study center by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of subjects by the Investigator.
- Discontinuation of further study treatment development.

Publication Policy

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and unethical practice. Novus Therapeutics, Inc, as the Sponsor, has proprietary interest in the study 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 manuscript 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.

Appendix 3 Clinical Laboratory Tests

- The tests detailed in Table 8 will be performed by the central laboratory/by the local laboratory.
- Local laboratory results are only required in the event that the central laboratory results are not available in time for either study treatment administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either a study treatment decision or response evaluation, the results must be entered into the CRF.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5.0 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 8 Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters	
Hematology	Platelet Count	White Blood Cell Count with Differential:
	Red Blood Cell Count	Neutrophils
	Hemoglobin	Lymphocytes
	Hematocrit	Monocytes
	Red Blood Cell Indices:	Eosinophils
	Mean corpuscular volume (MCV)	Basophils
	Mean corpuscular hemoglobin (MCH)	
	Mean cell hemoglobin concentration (MCHC)	
	%Reticulocytes	
Clinical Chemistry	Blood urea nitrogen (BUN)	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)
	Creatinine	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)
	Glucose	Alkaline phosphatase (ALP)
	Gamma glutamyl transferase (GGT)	Creatine kinase
	Magnesium	Creatine kinase MB fraction will be performed if clinically indicated
	Cholesterol	Chloride
	Potassium	Globulin
	Sodium	Amylase
	Calcium	Total and direct bilirubin

Laboratory Assessments	Parameters	
	Lactate dehydrogenase	Conjugated and unconjugated bilirubin will be performed if clinically indicated
	Albumin	Total Protein
	Uric acid	Phosphate
	C-reactive protein	Carbon dioxide (bicarbonate)
	Triglycerides	
Coagulation	International normalized ratio (INR)	Activated partial thromboplastin time (APTT)
	Prothrombin time (PT)	Thrombin time
	Partial prothrombin time	Fibrinogen
Urinalysis	Leucocytes	Red blood cells
	Protein	pH
	Bilirubin	Nitrite
	Urobilinogen	Specific gravity
	Ketones	Glucose
	Microscopy (if clinically indicated)	
Viral serology	HIV I and II	Hepatitis C Virus
	HBsAg	
Urine drug screen	Amphetamine	Opiates
	Ethanol	Benzodiazepines
	Cannabinoids	Methadone metabolites
	Cocaine metabolites	Barbiturates
	Urine creatinine	Ecstasy (3,4-Methylenedioxymethamphetamine)
	Cotinine	Phencyclidine
	Tricyclin antidepressants (TCA)	
Other screening tests	Follicle stimulating hormone (postmenopausal women only)	
	Serum or urine human chorionic gonadotropin (hCG) pregnancy test for all women[a]	

NOTES:
a Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

Investigators must document their review of each laboratory safety report.

Appendix 4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgement of the Investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded as pre-treatment-emergent AEs in the AE section of the eCRF. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a) Results in death
b) Is life-threatening The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c) Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d) Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e) Is a congenital anomaly/birth defect
f) Other situations: • Medical or scientific judgement should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording and Follow-up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the CRF. Each event must be recorded separately.
- It is **not** acceptable for the Investigator to send photocopies of the subject's medical records in lieu of completion of the AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

The Investigator must assess the causality of an AE (including SAEs) to the use of a study drug using a 2-category scale (not related, or related) based on clinical judgement and using all available information, and may include consideration of the following factors:

- Possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors.
- The temporal association between drug exposure and onset of the AE.
- Whether the manifestations of the AE are consistent with known actions or toxicity of the investigational product.
- Whether the AE resolved or improved with stopping use of the investigational product. Judgement should be used if multiple products are discontinued at the same time.
- Positive rechallenge.
- Positive dechallenge (resolution upon stopping suspect the IP, in absence of other intervention or treatment).
- The causal relationship between at study drug and the AE will be assessed using one of the following categories:
    - **Not Related**: An AE is not associated with study medication if no causal relationship exists between the IP and the AE, but an obvious alternative cause exists, eg, the subject's underlying medical condition or concomitant therapy.
    - **Related**: An AE is attributed to the study medication if there is reasonable/plausible possibility that the AE may have been caused by the IP.

Follow-up of AEs and SAEs

- 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.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Reporting of SAEs

SAE Reporting via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE will be the electronic data collection tool.
- If the electronic system is unavailable for more than 24 hours, then the study center will use the paper SAE data collection tool (see next section).
- The study center will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given study center, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a study center receives a report of a new SAE from a subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the study center can report this information on a paper SAE form (see next section) or to the Medical Monitor/SAE coordinator by telephone.

SAE Reporting via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor or the SAE coordinator. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE CRF pages within the designated reporting time frames.

Appendix 5 Excluded Medications/Therapy

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

- Medications (either topically or systemically) for a condition related to ear or nose.
- Anti-cholinergic medications (eg, anti-histamine, anti-nausea).
- Any prescription drug (except oral contraceptives, See Appendix 6).
- Administration of any intranasal product other than the Investigational Product.

Appendix 6 Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of childbearing potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   a) Documented hysterectomy.
   b) Documented bilateral salpingectomy.
   c) Documented bilateral oophorectomy.
   Note: Documentation can come from the study center personnel's: review of the subject's medical records, medical examination, or medical history interview.
3. Postmenopausal female:
   a) A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
   b) Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male subjects

- Male subjects with female partners of childbearing potential are eligible to participate if they agree to ONE of the following:
  - Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent for duration of study.
  - Agree to use a male condom and have their partner use of a contraceptive method with a failure rate of <1% per year as described in the table below when having penile-vaginal intercourse with a WOCBP who is not currently pregnant.

- In addition, male subjects must refrain from donating sperm for the duration of the study and for 90 days following the last administration of study treatment.
- Male subjects with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female subjects

Female subjects of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in the table below for the duration of the study and for 30 days following the last dose of study treatment.

Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral. • Intravaginal. • Transdermal.
Progestogen only hormonal contraception associated with inhibition of ovulation • Oral. • Injectable.
Highly Effective Methods That Are User Independent [a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD). • Intrauterine hormone-releasing system (IUS). Bilateral tubal occlusion.
Vasectomized Partner *A vasectomized partner is a highly effective birth control method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual Abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*
NOTES: [a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies. [b] Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period.

Pregnancy Testing:

- WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test.
- Additional pregnancy testing should be performed at times specified in the SoA during the treatment period.

Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

Collection of Pregnancy Information

Male subjects with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive study treatment.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Subjects who become pregnant

- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy related SAE considered reasonably related to the study treatment by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former subjects, he or she may learn of an SAE through spontaneous reporting.

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

Continuation of study treatment may only be allowed if either of the following criteria is met:

The study treatment has an approved label that indicates it can be used safely in pregnant females.

OR

All of the following apply:

- The subject has a high mortality disease.
- The Investigator determines the subject is benefitting from study participation and there is no other reasonable treatment for her.
- The Sponsor and the relevant IRB/IEC give written approval.
- The subject gives signed informed consent.
- The Investigator agrees to monitor the outcome of the pregnancy and the status of the subject and her offspring.
- The protocol is amended to allow such participation on a case-by-case basis, if such participation is not already addressed in the protocol.

Appendix 7 Device Experience Questionnaire

[redacted]

For Clinical Staff

Thank you for completing the study activity.
Please rate your agreement to the following statements and circle your response.

1. **My first reaction to the study inhaler was positive.**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

2. **The study inhaler Instructions for Use were clear and easy to follow.**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

3. **If I was not instructed, I feel confident that I could have used the product without instruction**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

4. **The study inhaler is easy to use.**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

5. **It was easy to place the study inhaler in the nostril.**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

6. **It was easy to press down on the study inhaler.**

   Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

7. **Other comments.**

Thank you, please hand the questionnaire back to the Principle Investigator.

Site _____ Subject _____ Date _____

[redacted]

For Trial Subjects

Thank you for completing the study activity.

Please rate your agreement to the following statements and circle your response.

1. **My first reaction to the study inhaler was positive.**

Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

Comments:

2. **The study inhaler was comfortable in my nostril.**

Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

Comments:

3. **The spray had a pleasant taste or aroma.**

Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

Comments:

4. **There was no discomfort or irritation from the spray inside of the nose.**

Strongly Disagree | Disagree | Neutral | Agree | Strongly Agree

Comments:

5. **Other comments.**

Thank you, please hand the questionnaire back to the interviewer.

Site ____ Subject ____ Date ____

Appendix 8 Liver Safety: Suggested Actions and Follow-up Assessments

Not applicable.

Appendix 9 Medical Device Incidents: Definition and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definitions of a Medical Device Incident

The detection and documentation procedures described in this protocol apply to all Sponsor medical devices provided for use in the study (see Section 6.1) for the list of Sponsor medical devices).

Medical Device Incident definition

- A Medical Device Incident is any malfunction or deterioration in the characteristics and/or performance of a device as well as any inadequacy in the labeling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a subject/user/other person or to a serious deterioration in his/her state of health.
- Not all incidents lead to death or serious deterioration in health. The nonoccurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.

It is sufficient that:

- An **incident** associated with a device happened.

  AND

- The **incident** was such that, if it occurred again, might lead to death or a serious deterioration in health.

A serious deterioration in state of health can include any of the following:

- Life-threatening illness.
- Permanent impairment of body function or permanent damage to body structure.
- Condition necessitating medical or surgical intervention to prevent 1 of the above.
- Fetal distress, fetal death, or any congenital abnormality or birth defects.

Examples of incidents

- A subject, user, caregiver, or healthcare professional is injured as a result of a medical device failure or its misuse.
- A subject's study treatment is interrupted or compromised by a medical device failure.
- A misdiagnosis due to medical device failure leads to inappropriate treatment.
- A subject's health deteriorates due to medical device failure.

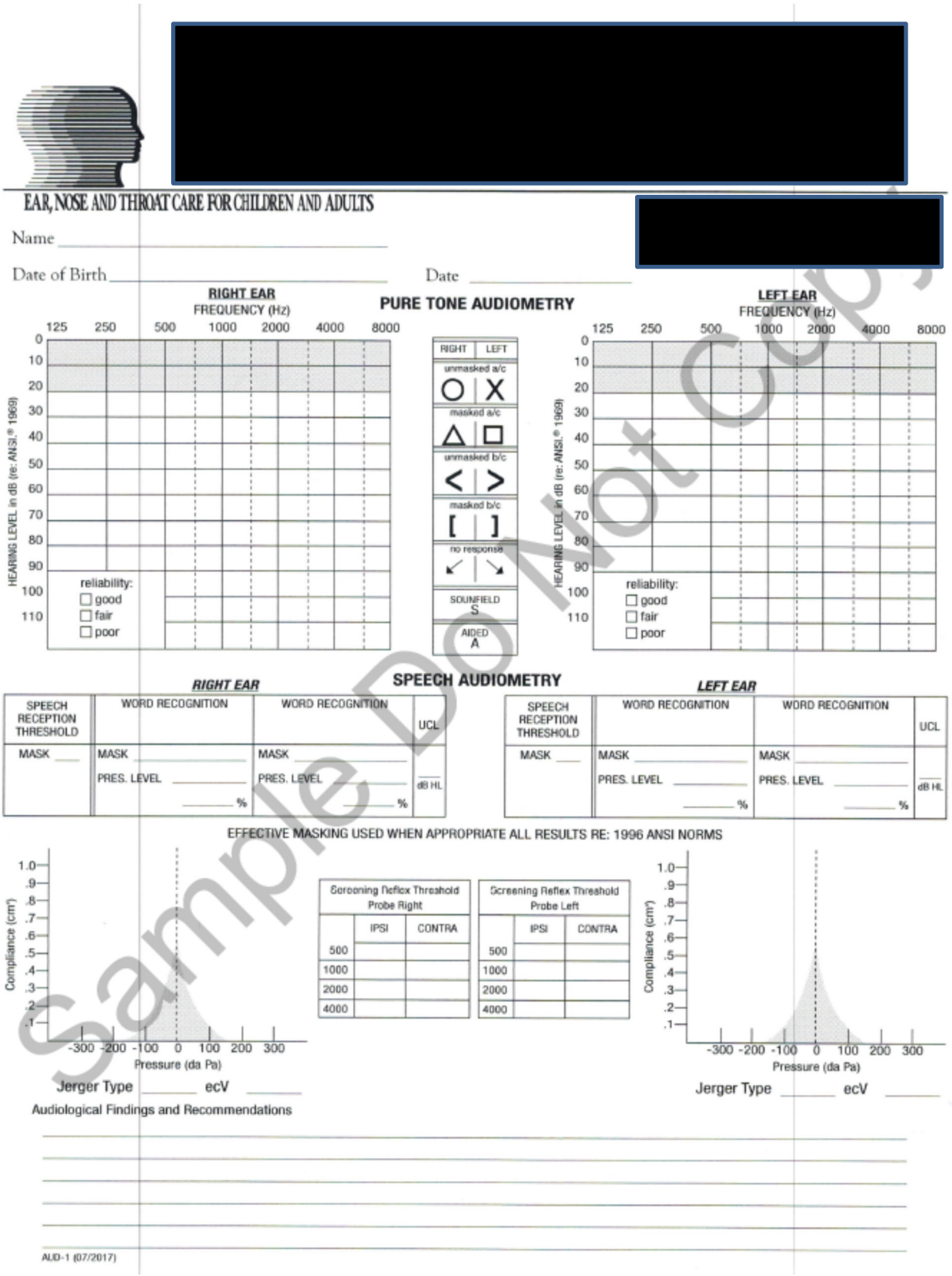
Documenting Medical Device Incidents

Medical Device Incident documenting

- Any Medical Device Incident occurring during the study will be documented in the subject's medical records, in accordance with the Investigator's normal clinical practice, and on the appropriate form of the CRF.
- For incidents fulfilling the definition of an AE or an SAE, the appropriate AE/SAE CRF page will be completed as described in Appendix 4.
- The CRF will be completed as thoroughly as possible and signed by the Investigator before transmittal to the Sponsor or designee.
- It is very important that the Investigator provides his/her assessment of causality (relationship to the medical device provided by the Sponsor) at the time of the initial AE or SAE report and describes any corrective or remedial actions taken to prevent recurrence of the incident.

A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.

Appendix 10 Audiology Testing Template



Appendix 11 Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

Appendix 12 Signature of Investigator

PROTOCOL TITLE: Evaluation of safety of repeated doses of OP0201 metered dose inhaler compared to placebo in healthy adult volunteers

PROTOCOL NO: OP0201-C-002

VERSION: Amendment 1

This protocol is a confidential communication of the Sponsor. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with GCPs and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to Sponsor or CRO.

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Center: _____

_____

_____