PMI RESEARCH & DEVELOPMENT

Clinical Study Protocol

Study title:	A 2-arm, open label, prefatory study to explore changes in nasal mucociliary clearance between smokers and never smokers and to standardize nasal scraping procedure.
Study number	P1-CMS-01-US
Short title	Prefatory study to explore changes in nasal mucociliary clearance and to standardize nasal scraping procedure.
EUDRACT number:	Not applicable.
Product name:	Not applicable
Sponsor:	Philip Morris Product S.A. PMI Research & Development Quai Jeanrenaud 5 2000 Neuchâtel, Switzerland
Version number:	Final 2.0 Amendment 1.0
Revision date:	22-May-2017
Authors:	[redacted]

PROTOCOL REVISION HISTORY

The following summarizes the updates made from Final Version 1.0 (8-Dec-2016) to Final Version 2.0 Amendment 1.0 (22-May-2017)

Synopsis – Study Population and Main Criteria for Inclusion/Exclusion Section	
Inclusion criteria 2. updated **from**: • Male subject aged ≥25 to ≤40 years old. Rationale for change: Sponsor decision to widen the age range of enrolled subjects.	**To:** • Male subject aged ≥25 to ≤45 years old.
Section 5.1 Selection of Study Population	
Inclusion criteria 2. updated **from**: • Male subject aged ≥25 to ≤40 years old. Rationale for change: Sponsor decision to widen the age range of enrolled subjects.	**To:** • Male subject aged ≥25 to ≤45 years old.
Appendix B – Inclusion and Exclusion Criteria Assessment	
Inclusion criteria 2. updated **from**: • Male subject aged ≥25 to ≤40 years old. Rationale for change: Sponsor decision to widen the age range of enrolled subjects.	**To:** • Male subject aged ≥25 to ≤45 years old.

PROTOCOL AUTHORISATION

Study Title: A 2-arm, open label, prefatory study to explore changes in nasal mucociliary clearance between smokers and never smokers and to standardize nasal scraping procedure.

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the methods to be used, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the current Declaration of Helsinki and the guidelines on Good Clinical Practice.

LOYSE FELBER MEDLIN

Loyse Felber Medlin, PhD, Clinical Scientist (Print)

[redacted]

Loyse Felber Medlin, PhD, Clinical Scientist (Signature)

22-MAY-2017

Date

Gizelle Baker

Gizelle Baker, PhD, Study Statistician (Print)

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22 may 2017

Date

NICOLAS BLANC

Nicolas Blanc, MD, Medical Safety Officer (Print)

[redacted]

Nicolas Blanc, MD, Medical Safety Officer (Signature)

22 - May - 2017

Date

DECLARATION OF INVESTIGATOR

Study Title: A 2-arm, open label, prefatory study to explore changes in nasal mucociliary clearance between smokers and never smokers and to standardize nasal scraping procedure.

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, case report forms (CRFs), and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB). No substantial changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB, except where necessary to avert an immediate hazard to the subjects.

I have read the protocol and agree that the study will be conducted in compliance with the protocol and in accordance with the principles of the current version of the Declaration of Helsinki (Recommendations guiding Medical Doctors in Biomedical Research Involving Human Subjects). The conduct of the study will be in accordance with the Notes for Guidance on Good Clinical Practice (GCP) (CPMP/ICH/135/95).

I acknowledge that I am responsible for the overall study conduct. I agree to personally conduct or supervise the described clinical study. I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study at my site are informed about their obligations. Mechanisms are in place to ensure that site staff receive the appropriate information throughout the study.

	Neptune, NJ, USA
Frank Lee, MD, Investigator (Print)	Investigational Site
Frank Lee, MD, Investigator (Signature)	Date

SYNOPSIS

Sponsor:

Philip Morris Product S.A.
Quai Jeanrenaud 5
2000 Neuchâtel
Switzerland

Name of Product:

Not applicable.

Study Title:

A 2-arm, open label, prefatory study to explore changes in nasal mucociliary clearance between smokers and never smokers and to standardize nasal scraping procedure

Study Number:

P1-CMS-01-US

Inflamax study code: 15-LE-001-RD

Study Short Title:

Prefatory study to explore changes in nasal mucociliary clearance and to standardize nasal scraping procedure.

Study Location:

Objectives and endpoints:

Primary Objective and Endpoints:

1. To evaluate the NMC over the course of 12 hours following single use of cigarette in smokers.
- STT value as assessed by STT test at each time point.

2. To compare NMC over the course of 12 hours in smokers following single use of cigarette relative to never smokers.
- STT value as assessed by STT test at each time point.

3. To examine the relationship between plasma nicotine levels and STT value in smokers and never smokers.
- STT value as assed by the STT test and plasma nicotine levels at each time point.

Secondary Objectives and Endpoints:

1. To standardize nasal scraping procedure using two methods.
- Collection of nasal epithelium for further histology.
- Evaluation of RNA quality and quantity.

2. To monitor the safety during the study.
- Vital signs.
- Adverse events (AE).
- Nasal and throat exam.
- Hematology, clinical chemistry and urine analysis.
- Brief physical examination.
- Concomitant medications.

Additional Study Assessments (for baseline characteristics):

- Serology for human immunodeficiency virus (HIV) 1/2 and hepatitis B and C.
- Alcohol breath test.

Evaluation Criterion:

The evaluation criterion is the time lapse between placing the saccharin and tasting the sweetness. These times will be evaluated by the summary statistics described elsewhere.

Study Hypothesis:

There are no statistical hypotheses to be tested.

Study Design:

This will be a single-center, study in which 14 healthy adult male study participants, consisting of 7 cigarette smokers and 7 never smokers as a control group, will be enrolled. Study participants will not be replaced after being enrolled.

The subjects will be divided in two groups:

Group 1 – 7 cigarette smokers

Group 2 – 7 never smokers

This study will have three visits on three separate days as described in Figure 1.

At Visit 1 (Screening Visit), study participants will undergo the procedures outlined in Appendix A to check eligibility criteria, including providing informed consent, undergoing a brief physical examination, nasal and throat exam, vital signs, skin prick test (SPT), spirometry testing, STT test, exhaled carbon monoxide (eCO) measurements, urine cotinine assessment, medical and smoking history and blood and urine collection for medical lab exams.

Following Visit 1, study participants who pass all of the inclusion criteria and do not present any of the exclusion criteria will be asked to return to the clinic for Visit 2.

During Visit 2, selected inclusion and exclusion criteria will be reassessed to ensure study participants are still eligible for enrollment (Appendix A). All study participants will have baseline assessments performed (Appendix A), including STT test.

Study participants will be confined in the clinic from the beginning of Visit 2 until discharge at Visit 3. During this confinement period study participants will abstain from drinking any alcohol and any drinks containing caffeine. Standard meals will be served. Drug restrictions applicable to study participant exclusion will also be maintained until the end of study (EOS).

No cigarettes are to be smoked from midnight (± 1 hour) between Visit 2 and Visit 3 until 8 am on the day of Visit 3.

On the day of Visit 3, at least 8 hours after last cigarette, cigarette smokers, who want to smoke, will have a cigarette at 8 am ± 30 minutes and then have the STT test performed immediately after smoking (5-10 minutes post T_0). T_0 will be defined as the start of cigarette smoking for smokers. T_0 should be the same for all smokers within an acceptable time window of 1 hour. Never smokers will also have the STT test performed in the morning at 8 am ± 30 minutes (designated $T_0 \pm 30$ minutes).

All study participants (smokers and never smokers) will then have the STT test performed at 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0, ensuring at least approximately 4 hours between the beginning of each STT test. The participant will be asked to confirm he can no longer feel the sweet taste. Study participants will drink a glass of water (approximately 240 mL) before STT tests are performed.

Samples for plasma nicotine assessment will be collected at approximately the same time points that STT test is performed at T_0, 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0.

Nasal scraping of the inferior turbinate of both nostrils will be performed on all 14 study participants at the end of Visit 3, after all other clinic assessments, except the brief physical examination which will be done after the nasal scraping:

- For 4 study participants, nasal scraping (left nostril with method 1, right with method 2) will be collected and shipped to the appropriate laboratory for further histological assessment.

- For the 10 remaining study participants, 5 will undergo nasal scraping using method 1, and 5 using method 2. For all 10 study participants, cell sample from one nostril will be added into one buffer and cell sample from the other nostril into a second buffer. Samples will be collected and shipped to the appropriate laboratory for subsequent RNA quantity and quality analysis.

Full procedure is described in the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe".

Upon conclusion of all clinic assessments, at the time of discharge on Visit 3, the Principal Investigator or designee will perform a brief physical examination on the study participant.

From the discharge on Visit 3, the study participant will enter a 1-day Safety Follow-Up Period during which spontaneously reported new AEs/serious AEs (SAEs) will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site.

Any non-serious AE that is ongoing during the Safety Follow-Up Period will be actively followed-up by the Principal Investigator or designee during that period until it has been resolved, stabilized (i.e., no worsening of the condition), an acceptable explanation has been found (e.g., a chronic condition) or lost to follow up.

At the end of the Safety Follow-Up Period, all ongoing non-serious AEs will be documented as "ongoing" and no follow-up information will be sought for on them anymore by the Principal Investigator or designee. At that point, the Investigator will assess whether the subject should be referred to his/her General Practicioner to have their ongoing AEs addressed accordingly.

All SAEs will be followed up by the Investigator or designee, despite their continuation after the end of the Safety Follow-Up Period, until their resolution, stabilization (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

Subjects who are discontinued from the study because of an AE will undergo the early termination procedures as soon as practical after the day of discontinuation and will enter the period of safety follow-up.

The end of study for a study participant is defined as the date of discharge on Visit 3 plus one day of Safety Follow-Up Period or the date of early termination of the study participant plus one day of Safety Follow-Up Period.

The end of the entire study is defined as the end of the Safety Follow-Up Period of the last study participant.

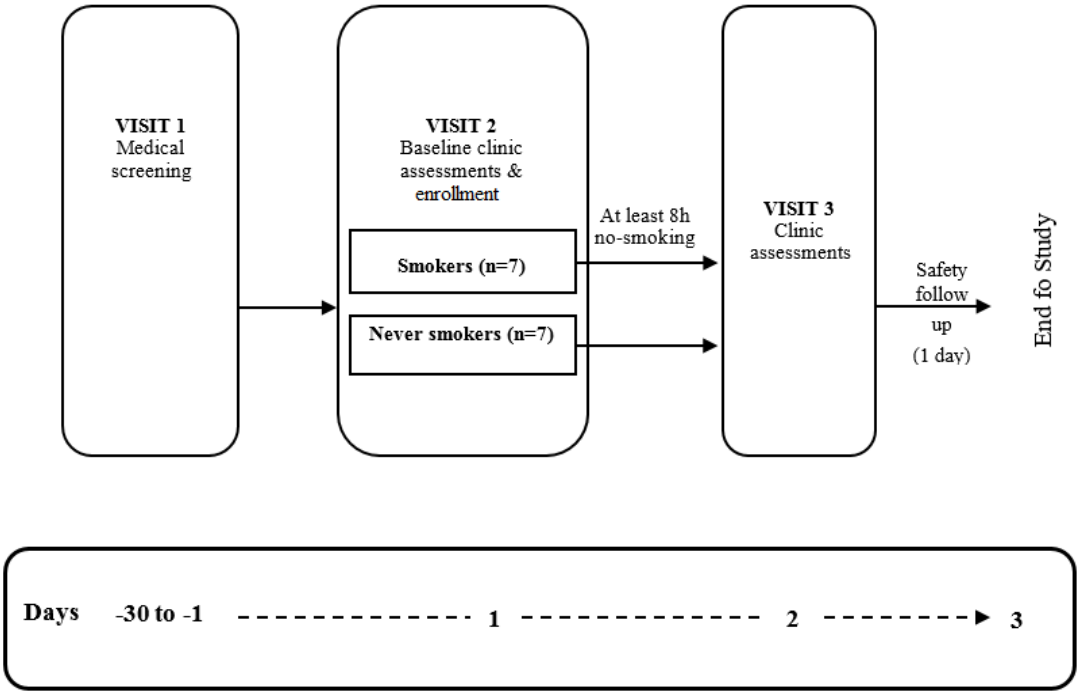

Figure 1 Study Flow Chart

Study Population and Main Criteria for Inclusion/Exclusion:

Inclusion criteria:

1. Informed of the nature of the study and have agreed to and are able to read, review, and sign the informed consent form (ICF) prior to Screening. The subject must be willing to comply with the study procedures described in the informed consent. The informed consent document will be written in English, therefore the volunteer must have the ability to read and communicate in English.
2. Male subject aged ≥25 to ≤45 years old.
3. BMI between 18.0 kg/m^2 to 32.0 kg/m^2, inclusive.
4. Judged by the Principal Investigator or designee to be in good health as documented by the medical history, physical examination, vital sign assessments, clinical laboratory assessments, and by general observations.
5. Belong to one of the following two groups:
    a. Non-menthol cigarette smoker (meets all of the following criteria at Visit 1 and at Visit 2):
        i. A positive urine cotinine test (≥200 ng/mL).
        ii. Smoked at least 20 cigarettes per day for at least the past 5 years.
        iii. eCO levels >10 parts per million (ppm).
        iv. No plans to quit smoking in the next 3 months.
    b. Never smoker (meets all of the following criteria at Visit 1 and at Visit 2):
        i. Subject who has smoked less than 100 cigarettes throughout their lifetime and no cigarettes in the past 3 years.
        ii. A negative urine cotinine test (<200 ng/mL).
        iii. eCO levels ≤ 5 ppm.
6. Completed the Screening process within 30 days prior to Visit 2.

7. Availability for the entire study period and willingness to comply with study procedures, including smoking interruptions, as evidenced by a signed ICF and at Visit 2.

Exclusion criteria:

1. As per the Principal Investigator or designee's judgment, the subject cannot participate in the study for any reason (e.g., medical, psychiatric, and/or social reason).
2. Subject is legally incompetent, or physically or mentally incapable of giving consent (e.g., emergency situation, under guardianship, prisoners, or subjects who are involuntarily incarcerated).
3. Presence of confounding allergies including allergic rhinitis and non-allergic rhinitis during the course of the study based on medical history and SPT.
4. Clinical significant abnormality on their nasal and throat exam, at the discretion of the Principal Investigator or designee at Visit 1 and/or at Visit 2.
5. Cigarette smoker who smoke/use any tobacco or nicotine products (other than CC), such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 months, as self-reported at Visit 1 or Visit 2.
6. Never smoker who smoke/use any tobacco or nicotine products, such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 years, as self-reported at Visit 1 or Visit 2.
7. Cigarette smokers who state they will be unable to abstain from smoking for up to 24 hours.
8. Inability to taste sweet within 60 minutes in the STT test.
9. Subjects who routinely use or who have used in the previous 4 weeks nasal sprays, inhalers or other nasal products, such as nasal irrigation (for example, Neti Pot) prior to Visit 1 and/or Visit 2.
10. Subjects who have taken any of the following medication without the indicated minimum washout period:

Table 1 - Prohibited medication.

Prohibited Medication	Restriction period (with Principal Investigator or designee discretion)
Short-acting antihistamines including intranasal antihistamines	3 days before Visit 1 until Visit 2
Long-acting antihistamines (i.e. Loratadine, Desloratadine)	7 days before Visit 1 until Visit 2
Over-the-counter cough and cold preparations or sleep aids containing antihistamines	3 days before Visit 1 until Visit 2
Leukotriene inhibitors	14 days before Visit 1 until Visit 2
Oral or intra-articular steroid	30 days before Visit 1 until Visit 2
Intranasal and inhaled corticosteroids	14 days before Visit 1 until Visit 2
Use of monoamine oxidase inhibitors	14 days before Visit 1 until Visit 2
Decongestants	48 hours before Visit 1until Visit 2
Cromolyn products	14 days before Visit 1 until Visit 2
Beta-adrenergic blockers (i.e. Acebutolol, Atenolol, etc.)	14 days before Visit 1 until Visit 2
Anticholinergics	7 days before Visit 1 until Visit 2
Herbal or natural product remedies for allergy symptoms	On the day of Visit 1 until Visit 2
Short-Acting Beta Agonists	6 hours prior to spirometry (except as per protocol before spirometry)
Long-Acting Beta Agonists	3 days before Visit 1 until Visit 2
Phosphodiesterase 5 inhibitors (i.e. Sildenafil, Vardenafil, Tadalafil)	7 days before Visit 1 until Visit 2
Amiloride	3 days before Visit 1 until Visit 2
Macrolide antibiotics	7 days before Visit 1 until

	Visit 2
Guaifenesin	3 days before Visit 1 until Visit 2
Mucolytic agents	14 days before Visit 1 until Visit 2
Topical menthol products	14 days before Visit 1 until Visit 2
Topical nasal medication	4 weeks before Visit 1 until Visit 2
Topical ocular medication	4 weeks before Visit 1 until Visit 2
Any other medication at the Principal Investigator or designee's discretion that might interfere with the endpoints or procedures.	As per Principal Investigator or designee.

11. Subjects with evidence of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I or greater, and a forced expiratory volume 1 / the forced vital capacity ratio (FEV_1/FVC ratio) <0.7.
12. Any condition the Principal Investigator or designee has cause to believe would interfere with the procedures for upper or lower airway function. This could include, but is not limited to, nasal/septum deviations, or nasal polyps or nasal allergies which will be identified by the Principal Investigator or designee.
13. Upper or lower respiratory diseases in the 4 weeks prior to Visit 2.
14. History of nasal or sinus surgery in the 5 years prior to Visit 2.
15. As per the Principal Investigator or designee's judgment, the subject has medical conditions which require or will require in the course of the study, a medical intervention (e.g., start of treatment, surgery, hospitalization) which may interfere with the study participation and/or study results.
16. The subject has a positive alcohol test and/or a history of alcohol abuse that could interfere with the subject's participation in study at Visit 1 or Visit 2.
17. Positive urine drug screen at Visit 1 or Visit 2.

18. The subject has positive serology test for human immunodeficiency virus (HIV)1/2, Hepatitis B or Hepatitis C.
19. Subject has donated or been in receipt of whole blood or blood products within 3 months prior to Visit 1.
20. Subject is a current or former employee of the tobacco industry or of their first-degree relatives (spouse, legal patner, parent, sibling, and child).
21. Subject is an employee of the investigational site or any other parties involved in the study or of their first-degree relatives (spouse, legal patner, parent, sibling, and child).
22. Subject has been in receipt of last dose from another clinical study within 3 months prior to Visit 1.
23. Subject has been previously screened in this study.

Investigational Product

Not applicable.

Duration of Study:

The planned maximum study duration for a single study participant from Screening through completion of study will be 33 days.

The estimated maximum study duration for the whole Study from screening through completion will be 35 days.

Statistical Methods

STT for each time point will be summarized by smoker and never smoker group using the descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

The plasma nicotine levels for each time point will be summarized by smoker and never smoker group using the descriptive statistics mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI). The relationship between plasma nicotine levels and STT at each time point will be evaluated by spearman correlation as well as graphical presentation of these endpoints by time point and smoker and never smoker group.

An overall summary of AEs will be presented by smoker and never smoker group showing the number of events and percent of study participants who experienced AEs, SAEs, severe AEs and AEs leading to study discontinuation.

RNA concentration will be summarized by method using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

RNA RIN will be summarized by method using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

A summary of AEs by System Organ Class (SOC) and Preferred Term (PT) by smoker and never smoker group will be presented.

Vital signs will be summarized by smoker and never smoker group using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

Sample Size

This is an exploratory study. This study will include 14 study participants: 7 cigarette smokers and 7 never smokers. No formal powering has occurred. However, with an expected standard deviation of 2.5, a difference in STT of 3.75 can be detected.

Human Subject Protection and Institutional Review Board (IRB):

CHESAPEAKE IRB

6940 Columbia Gateway Drive, Suite 110

Columbia, MD 21046-3403, USA

+1 410.884.2900

+1 410.884.9190

Safety reporting:

SAEs are reported from the time the study participants have signed their informed consent forms until the end of the study. The Principal Investigator or designee must notify sponsor of all SAEs cases to the sponsor representative within 24 hours of first awareness. Expedited reporting of SAEs to IRB(s) and/or competent authorities will be done as locally required.

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
ATS	American Thoracic Society
BMI	Body mass index
BUN	Blood urea nitrogen
CF	Cystic fibrosis
CFR	Code of Federal Regulation
CI	Confidence interval
COHb	Carboxyhemoglobin
COPD	Chronic pulmonary obstructive disease
CRF	Case report form
CRO	Contract research organization
CSR	Clinical study report
CV	Curriculum vitae
DMP	Data management plan
DNA	Deoxyribonucleic acid
eCO	Exhaled carbon monoxide
EOS	End of study
FAS	Full analysis set
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume 1
FVC	Forced vital capacity
GCP	Good Clinical Practice

GGT	Gamma-glutamyl transferase
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council for Harmonization
IRB	Institutional Review Board
IU	International units
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantification
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDI	Metered dose inhaler
MedDRA	Medical dictionary for regulatory activities
NMC	Nasal mucociliary clearance
PMI	Philip Morris International
PP	Per protocol
ppm	Parts per million
PT	Preferred term
QC	Quality control
RBC	Red blood cell
RNA	Ribonucleic acid
RIN	RNA integrity Number
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
SOP	Standard operating procedure

SPT	Skin prick test
STT	Saccharin transit time
T_0	Time point of first product use during study day
TLC	Total lung capacity
UBC	United BioSource Corporation
WBC	White blood cell

Explanation of Terms

The following special terms are used in this protocol:

Cigarette	The term 'cigarette' refers to commercially available cigarettes (manufactured) and excludes cigars, pipes, bidis, and other nicotine-containing products.
End of study (EOS)	The EOS for a study participant is defined as Discharge on Visit 3 plus the 1 day for the Safety Follow-up Period or the date of early termination of the study participant plus the 1 day for the Safety Follow-up Period. The EOS of the entire study is the end of the Safety Follow-up Period of the last study participant.
Screening failure	All screened study participants that are not enrolled because the fail to comply with inclusion/exclusion criteria are considered as screen failures.

1 ETHICS AND REGULATIONS

1.1 Institutional Review Board (IRB) Approval

Prior to the start of the study, the clinical study protocol, together with its associated documents (Informed Consent Form (ICF), subject information sheet, subject recruitment procedures (*e.g.*, advertisements), written information to be provided to the subjects, available safety information, the Principal Investigator's curriculum vitae (CV) and/or other evidence of qualifications and any other documents requested by the Institutional Review Board (IRB), will be submitted for review and approval to the relevant IRB. The IRB shall be appropriately constituted and perform its functions in accordance with the International Conference on Harmonization (ICH) Tripartite Guidance for Good Clinical Practices (GCPs) and local requirements, as applicable.

The written approval from the IRB will be filed in the Investigator Site File, and a copy will be filed in the study master file at the Sponsor or designated organization. The study must not start at a site before the Sponsor has obtained written confirmation of favorable opinion/approval from the concerned IRB.

Any substantial change or addition to this protocol will require a written protocol amendment that must be approved by the Sponsor and the Principal Investigator. All amendments will be submitted to the IRB, and substantial amendments will only be implemented after approval by the IRB. For modifications to the protocol which are administrative in nature, or do not affect subject risk, the IRB will be notified in writing by the Principal Investigator or designee with a copy provided to the Sponsor.

These requirements for approval should in no way prevent any action from being taken by the Principal Investigator or designee or by the Sponsor in order to eliminate immediate hazards to the subjects. If such a change to the protocol is felt to be necessary by the Principal Investigator or designee, and is implemented for safety reasons, the Sponsor and the IRB should be informed immediately.

The Principal Investigator is responsible for local reporting (*e.g.*, to the IRB) of SAEs that occur during the study, according to local regulations.

Relevant safety information will be submitted to the IRB during the course of the study in accordance with national regulations and requirements.

Medically qualified study personnel will be available during the study.

The following IRB will be used:

CHESAPEAKE IRB

6940 Columbia Gateway Drive, Suite 110

Columbia, MD 21046-3403, USA

+1 410.884.2900

+1 410.884.9190

1.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (World Medical Association, 2013) and is consistent with ICH/GCPs applicable regulatory principles.

The Principal Investigator or designee agrees to conduct the clinical study in compliance with the protocol agreed with the Sponsor and approved by the IRB. The Principal Investigator and the Sponsor must sign the protocol (and protocol amendments, if applicable) to confirm this agreement.

1.3 Subject Information and Consent

1.3.1 Informed Consent Form for Study Participation

Before or at Visit 1, the Principal Investigator or designee will ensure that each subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study, and the Principal Investigator or the designee will answer all questions the subject might have to his full satisfaction. The subject will have sufficient time for consideration of his participation in the study and will be notified that he is free to discontinue his participation at any time.

Once the subject has received all the necessary information, and if he agrees to participate, this will be documented in the ICF which includes both the subject information sheet and informed consent by the date, and signature of both the subject and the person who conducted the Inform Consent discussion during Visit 1. No study-specific procedures will be performed before the ICF has been signed and date and time will be recorded.

The original and completed ICF(s) must be kept by the Principal Investigator and filed in the Investigator study file at the site or with the study participant's files and a copy must be given to the study participant. The study participant will be informed that if he discontinues from the study, the data collected until the point of discontinuation will be maintained as part of the study data and the samples collected prior to discotinuation will be analyzed, unless he refuses it.

The subject will be informed that any analysis of the data will be covered by data confidentiality.

1.3.2 Amendment to the Informed Consent Form

If a protocol amendment is required, an amendment may be required to the ICF. If revision of the ICF is necessary, the Principal Investigator or designee will, with the support of the Sponsor, ensure that the documents have been reviewed and approved by the IRB before study participants are required to re-sign and date the ICF.

1.4 Good Clinical Practice and Regulatory Requirements

The procedures set out in this clinical study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Sponsor, its authorized representative, and Principal Investigator and designee abide by the principles of the ICH guidelines on GCPs. These guidelines apply specifically to pharmaceutical development, but nevertheless provide a robust and ethical framework for conducting clinical studies of tobacco products. The study will also be conducted in accordance with the general ethical principles outlined in the Declaration of Helsinki (World Medical Association, 2013).

In addition, the Principal Investigator or designee will carry out the clinical study in accordance with applicable national and local laws of the pertinent regulatory authorities.

2 INTRODUCTION

2.1 Background

Tobacco smoke is a complex mixture of chemical compounds that are bound to aerosol particles or exist free in a gaseous state. It has been estimated that cigarette smoke has more than 6000 chemical compounds from various classes, the majority of which upon combustion can transform and have great potential for toxic, harmful, oxidizing, and carcinogenic effects (FDA, 2010, 2014). Cigarette smoke has been linked to increased risk of chronic pulmonary obstructive disease (COPD), cardiovascular disease, and cancer, which results in more than 480,000 deaths each year in the United States alone, and 1.2 million deaths annually worldwide (CDC, 2015; FDA, 2010). Despite widespread knowledge of the health risk associated with cigarette smoking, it is estimated that the prevalence of this habit is in excess of 1 billion people worldwide (CDC, 2015).

Gaseous and particulate constituents of cigarette smoke first interface with the immune system at the mucosal surfaces lining the oral cavity, sinuses, and the airways (FDA, 2010; Hecht, 2003). As the inhaled cigarette smoke moves deeper into the respiratory tract, more soluble gases are absorbed and particulates are deposited into the lower airways (Hecht, 2003). The carcinogens and toxins produced by cigarette smoke can provoke an array of biological and immunological responses in the respiratory tract. With sustained exposure, these inhaled constituents can lead to carcinogenesis induction, non-resolving inflammation yet suppressive effects on immune cells, mucociliary impairment, and innate and adaptive immune dysfunction (Jaspers, 2014; Lee, Taneja, & Vassallo, 2012).

There are a number of mechanisms involved in protecting the respiratory tract from harmful substances such as cigarette smoke. Beating, synchronized cilia function is the first line of innate host defense by propelling foreign inhaled substances, microbes, and/or mucus, out of the upper airways via nasal mucociliary clearance (NMC) (Fahy & Dickey, 2010; Shah, Ben-Shahar, Moninger, Kline, & Welsh, 2009). Several factors affect NMC including aging, temperature, mucus pH, drugs, smoking, body mass index (BMI), allergic rhinitis, and sinusitis

(Baby, Muthu, Johnson, & Kannan, 2014; Özler, Şimşek, Akbay, & Akoğlu, 2014; Ramos et al., 2011, Paul 2016, Tamilselvan 2014, Balsamo 2010). A defect or impairment in ciliary motility, function, and consequently NMC, can cause increased airway inflammation, lung injury, bacterial colonization and eventually airway remodeling (Jain et al. 2012). Patients afflicted with diseases including cystic fibrosis (CF) and COPD have been shown to have impaired NMC function in their airway (Bhowmik, Chahal, Austin, & Chakravorty; Smaldone et al., 1993; Treacy, Tunney, Elborn, & Bradley, 2011). NMC measurement assesses the effectiveness of the interaction between cilia and mucus based on the measurement of (1) the transport of markers placed on the mucosa and (2) the total nasal clearance time of the deposited dose. There are a number of tracer molecules used to measure NMC time including saccharin, dye, and vegetable charcoal (Deborah & Prathibha, 2014). NMC strongly correlates with ciliary motility and function such that the greater the observed transit time, the further that ciliary motility and function is decreased (D. Passàli, Bellussi, & De Seta, 1984; D. Passàli & Bianchini, 1985; D. Passàli, Ferri, Becchini, Passàli, & Bellussi, 1999). Saccharin transit time (STT) is an inexpensive, non-invasive reproducible method that has been readily used to assess NMC (Stanley et al., 1984; Rutland & Cole, 1981). A positive correlation was observed between NMC, as determined by the STT test, and pulmonary clearance, measured by an inhaled radiolabelled aerosol (Loebinger, Bilton, & Wilson, 2009; Rutland & Cole, 1981).

Several authors have reported a decreased NMC in smokers versus non smokers and even passive smokers (Stanley et al., 1986, Habesoglu 2012, Utiyama et al., 2016). Moderate and heavy smokers show higher STT results than light smokers and non-smokers (Xavier et al., 2013). Decreased mucociliary function has been observed in people with moderate and heavy cigarette consumption (Proença et al., 2012). Smoking cessation appears to contribute favorably to mucociliary clearance (Ramos et al., 2011).

Proença et al. 2011 have studied the acute and short term effects of smoking on nasal mucociliary clearance in current smokers using the STT test immediately after smoking and 8 hours after the last cigarette. Smokers presented similar STT value immediately after smoking (11±6 min; $p = 0.87$) when compared to STT value in non-smokers' (10±4 min; mean±standard deviation), while the STT value in smokers 8 hours after smoking was significantly longer

(16±6 min; p = 0.005 versus non-smokers' and p = 0.003 versus immediately after smoking), (Proença et al., 2011). The alterations of the STT value over time are not fully clear, as was demonstrated in the above study where no baseline measurement was reported. Therefore, based on this, the need for the present prefatory study and its proposed design is justified. In order to better understand the difference in STT over time between smokers and never smokers, a never smoker group is added to the study design.

Additionally, samples from nasal scrapings using two methods will be collected during the study from smokers and never smokers. These two methods will be used in this prefatory study to identify and standardize the optimal one which results in the highest quantity and quality of ribonucleic acid (RNA) for further transcriptomics analysis. The use of multi-omics have been used to elucidate mechanistic pathways which are impacted by cigarette smoking. The transcriptome of multiple tissues are known to be altered due to cigarette smoke exposure. Through targeted transcriptomic analysis from nasal sampling, increased expression and identification of antioxidant and xenobiotic genes as well as a wide spectrum of inflammation-related genes can be characterized. (Martin, Talikka, Hoeng, & Peitsch, 2015; Phillips et al., 2016; Titz et al., 2016).

2.2 Purpose of the Study

This study intends to evaluate the NMC over the course of 12 hours following single use of cigarette in smokers, to compare NMC over the course of 12 hours in smokers following single use of cigarette relative to never smokers and to examine the relationship between plasma nicotine levels and STT value in smokers and never smokers.

Additionally it also intends to standardize a nasal scraping procedure using two methods with a histological assessment of nasal scraping and a RNA quality and quantity evaluation.

Safety will also be monitored during the study.

2.3 Anticipated Benefits and Risks

2.3.1 Anticipated Benefits

Subjects who participate in this study will benefit from a health check-ups, which may help to uncover undiagnosed medical conditions.

2.3.2 Anticipated Foreseeable Risks due to Study Procedures

The risk of procedures (*e.g.*, blood samples, nasal scrapes, spirometry) are deemed to be on par with procedures routinely performed during normal or extended health examinations by the study participant's health care professional. To monitor study participants for risks associated with study procedures after discharge, a follow-up period of 1 day is implemented.

2.3.3 Anticipated Foreseeable Risks due to Investigational Product

Not applicable.

3 STUDY OBJECTIVES

3.1 Primary Objectives and Endpoints

The primary objectives of this study are:

1. To evaluate the NMC over the course of 12 hours following single use of cigarette in smokers.

- STT value as assessed by STT test at each time point.

2. To compare NMC over the course of 12 hours in smokers following single use of cigarette relative to never smokers.

- STT value as assessed by STT test at each time point.

3. To examine the relationship between plasma nicotine levels and STT value in smokers and never smokers.

- STT value as assed by the STT test and plasma nicotine levels at each time point.

3.2 Secondary Objectives and Endpoints

The secondary objectives of this study are:

1. To standardize nasal scraping procedure using two methods .

- Collection of nasal epithelium for further histology .

- Evaluation of RNA quality and quantity.

2. To monitor the safety during the study.

- Vital signs.
- Adverse events (AE).
- Nasal and throat exam.
- Hematology, clinical chemistry and urine analysis.
- Brief physical examination.
- Concomitant medications.

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design and Plan

This will be a single-center study in which 14 healthy adult male study participants, consisting of 7 cigarette smokers and 7 never smokers as a control group, will be enrolled. Study participants will not be replaced after being enrolled.

This study will have three visits on three separate days as described in Figure 1.

At Visit 1 (Screening Visit), study participants will undergo the procedures outlined in Appendix A to check eligibility criteria, including providing informed consent, undergoing a brief physical examination (including an examination of the head and neck, chest, heart (cardiovascular) and abdomen), nasal and throat exam, vital signs, skin prick test (SPT), spirometry testing, STT test, exhaled carbon monoxide (eCO) measurements, urine cotinine assessment, medical and smoking history and blood and urine collection for medical lab exams.

Following Visit 1, study participants who pass all of the inclusion criteria and do not present any of the exclusion criteria will be asked to return to the clinic for Visit 2.

During Visit 2, selected inclusion and exclusion criteria will be reassessed to ensure study participants are still eligible for enrollment (Appendix A). All study participants will have baseline assessments performed (Appendix A), including STT test.

Study participants will be confined in the clinic from the beginning of Visit 2 until discharge at Visit 3. During this confinement period study participants will abstain from drinking any alcohol and any drinks containing caffeine.

Water will be allowed *ad libitum* except during STT tests. Throughout the study, standardized meals and beverages will be served. Meals will be the same in content and quantity during each confinement period. No food or drinks will be allowed during STT tests.

No cigarettes are to be smoked from midnight (± 1 hour) between Visit 2 and Visit 3 until 8 am on the day of Visit 3. On the day of Visit 3, at least 8 hours after last cigarette, cigarette smokers, who want to smoke, will have a cigarette at 8 am±30 minutes and then have the STT

test performed immediately after smoking (5-10 minutes post T_0). T_0 will be defined as the start of cigarette smoking for smokers (time point of first product use during study day). T_0 should be the same for all smokers within an acceptable time window of 1 hour. Never smokers will also have the STT test performed in the morning at 8 am ± 30 minutes (designated T_0 ± 30 minutes). All study participants (smokers and never smokers) will then have the STT test performed at 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0, ensuring at least approximately 4 hours between the beginning of each STT test. The participant will be asked to confirm he can no longer feel the sweet taste. Study participants will drink a glass of water (approximately 240 mL) before STT tests are performed.

Samples for plasma nicotine assessment will be collected at approximately the same time points that STT test is performed at T_0 , 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0.

Nasal scraping of the inferior turbinate of both nostrils will be performed on all 14 study participants at the end of Visit 3, after all other clinic assessments, except the brief physical examination which will be done after the nasal scraping:

- For 4 study participants, the nasal scraping (left nostril with method 1, right with method 2) will be performed and samples collected for subsequent histological assessment.

- For the 10 remaining study participants, 5 will undergo nasal scraping using method 1, and 5 using method 2. For all 10 study participants, cells from one nostril will be added into one buffer, cells from the other nostril into a second buffer. Samples will be collected for subsequent RNA quantity and quality analysis.

Full procedure is described in the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe".

Upon conclusion of all clinic assessments, at the time of discharge on Visit 3, the Principal Investigator or designee will perform a brief physical examination on the study participant.

From the discharge on Visit 3, the study participant will enter a 1-day Safety Follow-Up Period during which spontaneously reported new AEs/serious AEs (SAEs) will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site.

Any non-serious AE that is ongoing during the Safety Follow-Up Period will be actively followed-up by the Principal Investigator or designee during that period until it has been resolved, stabilized (i.e., no worsening of the condition), an acceptable explanation has been found (e.g., a chronic condition) or lost to follow up.

At the end of the Safety Follow-Up Period, all ongoing non-serious AEs will be documented as "ongoing" and no follow-up information will be sought for on them anymore by the Principal Investigator or designee. At that point, the Investigator will assess whether the subject should be referred to his/her General Practicioner to have their ongoing AEs addressed accordingly.

All SAEs will be followed up by the Investigator or designee, despite their continuation after the end of the Safety Follow-Up Period, until their resolution, stabilization (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

Subjects who are discontinued from the study because of an AE will undergo the early termination procedures as soon as practical after the day of discontinuation and will enter the period of safety follow-up.

The end of the study for a study participant is defined as the date of discharge on Visit 3 plus one day of Safety Follow-Up Period or the date of early termination of the study participant plus one day of Safety Follow-Up Period.

The end of the entire study is defined as the end of the Safety Follow-Up Period of the last study participant.

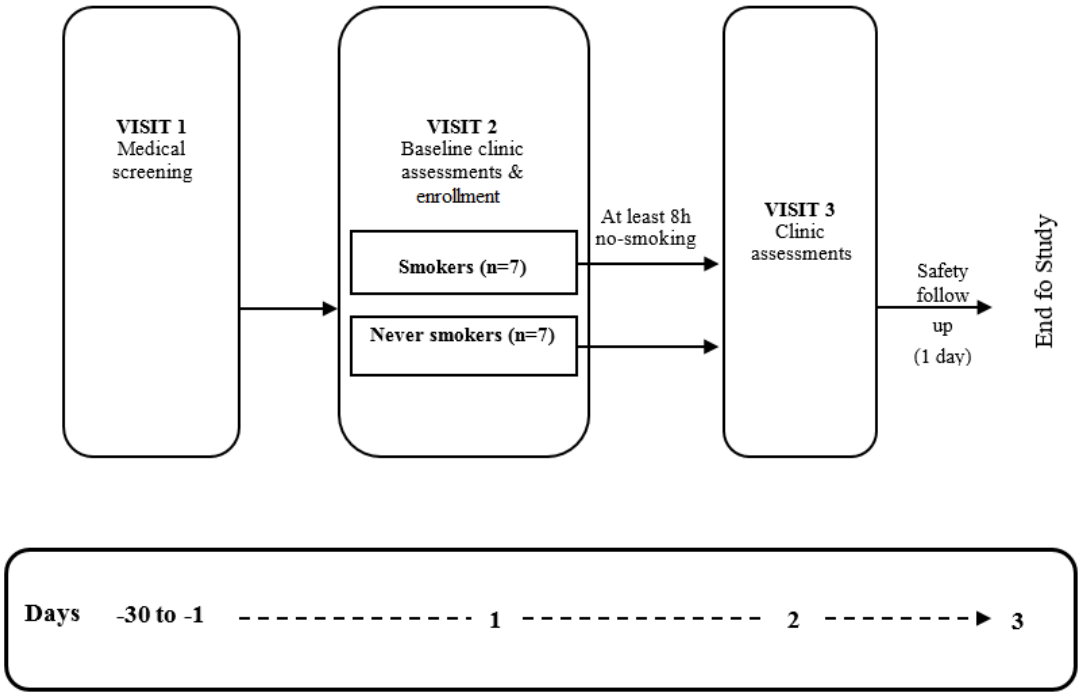

Figure 1 Study Flow Chart

4.2 Rationale for Study Design

Several authors have reported a decreased NMC in smokers versus non smokers and even passive smokers (Stanley et al., 1986, Habesoglu 2012, Utiyama et al., 2016). Moderate and heavy smokers show higher STT results than light smokers and non-smokers (Xavier et al., 2013). Decreased mucociliary function was observed in people with moderate and heavy cigarette consumption (Proença et al. 2012). Smoking cessation appears to contribute favorably to mucociliary clearance (Ramos et al. 2011).

Proença et al. 2011 have studied the acute and short term effects of smoking on nasal mucociliary clearance in current smokers using the STT test immediately after smoking and 8 hours after the last cigarette.

When compared to STT in non-smokers' (10±4 min; mean±standard deviation), smokers presented similar STT immediately after smoking (11±6 min; p = 0.87) and slower STT 8 hours after smoking (16±6 min; p = 0.005 *versus* non-smokers' and p = 0.003 *versus* immediately after smoking) (Proença et al., 2011). The alterations of the STT values over time is not fully clear as in the above study no baseline measurement has been reported and which establishes the need for the present prefatory study and justifies its proposed design. In order to better understand the difference in STT over time between smokers and never smokers, a never smoker group is added to the study design.

Additionally, samples from nasal scrapings using two methods will be collected during the study from smokers and never smokers. These two methods will be used in this prefatory study to identify and standardize the optimal one which results in the highest quantity and quality of ribonucleic acid (RNA) for further transcriptomics analysis. The use of multi-omics have been used to elucidate mechanistic pathways which are impacted by cigarette smoking. The transcriptome of multiple tissues are known to be altered due to cigarette smoke exposure. Through targeted transcriptomic analysis from nasal sampling, increased expression and identification of antioxidant and xenobiotic genes as well as a wide spectrum of inflammation-related genes can be characterized. (Martin, Talikka, Hoeng, & Peitsch, 2015; Phillips et al., 2016; Titz et al., 2016).

It was decided to focus the present study on a male population only to minimize the variability between genders.

4.3 Appropriateness of Measurements

STT measurements are an easy and effective method to measure NMC (Rutland and Cole 1981), whereby a 1-2 mm particle of saccharin is placed on the inferior nasal turbinate, 1 cm from the anterior end. The study participant will remain sitting for the duration of the test without speaking or engaging in any other activities that would disrupt the evaluation. The study participant will report when saccharin can be tasted and the time elapsed will be recorded and used as a measure of NMC (Rutland and Cole 1981). NMC strongly correlates with ciliary motility and function such that the greater the observed transit time, the further that ciliary motility and function is decreased (D. Passàli, Bellussi, & De Seta, 1984; D. Passàli & Bianchini, 1985; D. Passàli, Ferri, Becchini, Passàli, & Bellussi, 1999). The STT test has been

considered a "gold-standard" procedure for assessing NMC function as it permits large-scale assessment outside of specialized respiratory centres.

All laboratory measures utilized for this study are validated and are appropriate for the study assessments.

Since the test for nicotine concentration is highly sensitive, all study personnel who will be collecting blood must be non-smokers.

4.4 Study Duration

The planned maximum study duration for a single study participant from Screening through completion of study will be 33 days.

The estimated maximum study duration for the whole Study from screening through completion will be 35 days.

5 STUDY POPULATION

This study will enroll 14 study participants: 7 cigarette smokers and 7 never smokers.

The subjects will be divided in two groups:

Group 1 – 7 cigarette smokers

Group 2 – 7 never smokers

5.1 Selection of Study Population

An overview of the timing for assessment of each inclusion and exclusion criteria can be found in the Appendix B – Inclusion and Exclusion Criteria Assessment

5.1.1 Inclusion Criteria

Subjects who meet all the following inclusion criteria can be enrolled into the study:

1. Informed of the nature of the study and have agreed to and are able to read, review, and sign the informed consent form (ICF) prior to Screening. The subject must be willing to comply with the study procedures described in the informed consent. The informed consent document will be written in English, therefore the volunteer must have the ability to read and communicate in English.
2. Male subject aged ≥25 to ≤45 years old.
3. BMI between 18.0 kg/m^2 to 32.0 kg/m^2, inclusive.
4. Judged by the Principal Investigator or designee to be in good health as documented by the medical history, physical examination, vital sign assessments, clinical laboratory assessments, and by general observations.
5. Belong to one of the following two groups:
    a. Non-menthol cigarette smoker (meets all of the following criteria at Visit 1 and at Visit 2):

i. A positive urine cotinine test (≥200ng/mL).

ii. Smoked at least 20 cigarettes per day for at least the past 5 years.

iii. eCO levels >10 parts per million (ppm).

iv. No plans to quit smoking in the next 3 months.

b. Never smoker (meets all of the following criteria at Visit 1 and at Visit 2):

i. Subject who has smoked less than 100 cigarettes throughout their lifetime and no cigarettes in the past 3 years.

ii. A negative urine cotinine test (<200 ng/mL).

iii. eCO levels ≤ 5 ppm.

6. Completed the Screening process within 30 days prior to Visit 2.
7. Availability for the entire study period and willingness to comply with study procedures, including smoking interruptions, as evidenced by a signed ICF and at Visit 2.

5.1.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria must not be enrolled into the study:

1. As per the Principal Investigator or designee's judgment, the subject cannot participate in the study for any reason (e.g., medical, psychiatric, and/or social reason).
2. Subject is legally incompetent, or physically or mentally incapable of giving consent (e.g., emergency situation, under guardianship, prisoners, or subjects who are involuntarily incarcerated).
3. Presence of confounding allergies including allergic rhinitis and non-allergic rhinitis during the course of the study based on medical history and SPT.
4. Clinical significant abnormality on their nasal and throat exam, at the discretion of the Principal Investigator or designee at Visit 1 and/or at Visit 2.

5. Cigarette smoker who smoke/use any tobacco or nicotine products, such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 months, as self-reported at Visit 1 or Visit 2.
6. Never smoker who smoke/use any tobacco or nicotine products, such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 years, as self-reported at Visit 1 or Visit 2.
7. Cigarette smokers who state they will be unable to abstain from smoking for up to 24 hours.
8. Inability to taste sweet within 60 minutes in the STT test.
9. Subjects who routinely use or who have used in the previous 4 weeks nasal sprays, inhalers or other nasal products, such as nasal irrigation (for example, Neti Pot) prior to Visit 1 and/or Visit 2.
10. Subjects who have taken any of the following medication without the indicated minimum washout period:

Table 1 - Prohibited medication.

Prohibited Medication	Restriction period (with Principal Investigator or designee discretion)
Short-acting antihistamines including intranasal antihistamines	3 days before Visit 1 until Visit 2
Long-acting antihistamines (i.e. Loratadine, Desloratadine)	7 days before Visit 1 until Visit 2
Over-the-counter cough and cold preparations or sleep aids containing antihistamines	3 days before Visit 1 until Visit 2
Leukotriene inhibitors	14 days before Visit 1 until Visit 2
Oral or intra-articular steroid	30 days before Visit 1 until Visit 2
Intranasal and inhaled corticosteroids	14 days before Visit 1 until Visit 2
Use of monoamine oxidase inhibitors	14 days before Visit 1 until Visit 2
Decongestants	48 hours before Visit 1 until Visit 2

Cromolyn products	14 days before Visit 1 until Visit 2
Beta-adrenergic blockers (i.e. Acebutolol, Atenolol, etc.)	14 days before Visit 1 until Visit 2
Anticholinergics	7 days before Visit 1 until Visit 2
Herbal or natural product remedies for allergy symptoms	On the day of Visit 1 until Visit 2
Short-Acting Beta Agonists	6 hours prior to spirometry (except as per protocol before spirometry)
Long-Acting Beta Agonists	3 days before Visit 1 until Visit 2
Phosphodiesterase 5 inhibitors (i.e. Sildenafil, Vardenafil, Tadalafil)	7 days before Visit 1 until Visit 2
Amiloride	3 days before Visit 1 until Visit 2
Macrolide antibiotics	7 days before Visit 1 until Visit 2
Guaifenesin	3 days before Visit 1 until Visit 2
Mucolytic agents	14 days before Visit 1 until Visit 2
Topical menthol products	14 days before Visit 1 until Visit 2
Topical nasal medication	4 weeks before Visit 1 until Visit 2
Topical ocular medication	4 weeks before Visit 1 until Visit 2
Any other medication at the Principal Investigator or designee's discretion that might interfere with the endpoints or procedures.	As per Principal Investigator or designee.

11. Subjects with evidence of COPD, Global Initiative for Chronic Obstructive Lung Disease GOLD stage I or greater, and a forced expiratory volume 1 / the forced vital capacity ratio (FEV_1/FVC ratio) <0.7.

12. Any condition the Principal Investigator or designee has cause to believe would interfere with the procedures for upper or lower airway function. This could include, but is not limited to, nasal/septum deviations, or nasal polyps or nasal allergies which will be identified by the Principal Investigator or designee.
13. Upper or lower respiratory diseases in the 4 weeks prior to Visit 2.
14. History of nasal or sinus surgery in the 5 years prior to Visit 2.
15. As per the Principal Investigator or designee's judgment, the subject has medical conditions which require or will require in the course of the study, a medical intervention (e.g., start of treatment, surgery, hospitalization) which may interfere with the study participation and/or study results.
16. The subject has a positive alcohol test and/or a history of alcohol abuse that could interfere with the subject's participation in study at Visit 1 or Visit 2.
17. Positive urine drug screen at Visit 1 or Visit 2.
18. The subject has positive serology test for human immunodeficiency virus (HIV)1/2, Hepatitis B or Hepatitis C.
19. Subject has donated or been in receipt of whole blood or blood products within 3 months prior to Visit 1.
20. Subject is a current or former employee of the tobacco industry or of their first-degree relatives (spouse, legal partner, parent, sibling, and child).
21. Subject is an employee of the investigational site or any other parties involved in the study or of their first-degree relatives (spouse, legal partner, parent, sibling, and child).
22. Subject has been in receipt of last dose from another clinical study within 3 months prior to Visit 1.
23. Subject has been previously screened in this study.

5.2 Discontinuation of Study participants from the Study

Discontinued study participants will include both study participants who withdraw from the study (study participant's decision) or study participants who are removed from the study by the Principal Investigator. A study participant can only be discontinued from the study after enrollment.

Study participants will be informed that they are free to withdraw from the study at any time. Study participants should be questioned for the reason of premature withdrawal from the study, although they are not obliged to disclose it. This needs to be fully documented in the source document and case report form (CRF).

If the study participant withdraws his consent for the study, the Principal Investigator or designee needs to document if:

- The study participants agrees that the data can be used until the time of withdrawal.
- The study participant agrees to have his samples collected until the time of withdrawal to be analyzed.

This information needs to be fully documented in the source document and CRF.

When a study participant is discontinued from the study, he will be asked to perform the examination procedure planned in Section 9.4 as soon as possible after the time of discontinuation, unless the study participant is not willing to perform any additional assessment.

Study participants discontinued from the study cannot re-enter the study.

Study participants must be discontinued from the study for any of the following reasons:

- Withdrawal of informed consent.
- Any AE or condition (including clinically relevant changes in a laboratory parameter) which at the discretion of the Principal Investigator no longer justifies the study participant's participation in this study.
- The Sponsor or the Principal Investigator terminates the study. If the Sponsor or the Principal Investigator decides to prematurely terminate the study, the study participant

will be promptly informed. The Principal Investigator should report the fact and the reason of termination in writing to the IRB.

- Discontinuation is considered to be in the best interest for the study participant himself or for other study participants participating to the study.

Study participants may be discontinued from the study for any of the following reasons:

- Non-compliance to the study procedures based on the judgment of the Principal Investigator.

Study participants discontinued prematurely after enrollment will not be replaced and will not be allowed to re-enter the study. All study participant discontinuations must be documented properly in the source document and in the CRF.

5.3 Lost to Follow-up

Not applicable.

5.4 Violation of Selection Criteria

Study participants who are eligible at Screening (Visit 1), but who do not meet the entry criteria at Visit 2, will be considered as a screen failure until enrollment is completed.

If a violation of selection criteria after enrollment is detected, the study participant may be discontinued from the study at the discretion of the Principal Investigator or designee. If study participants are not yet enrolled, they can be replaced. If study participants are enrolled, and the Principal Investigator decides that they should be discontinued from the study, they will not be replaced.

6 INVESTIGATIONAL PRODUCTS

Not applicable.

6.1 Restrictions

6.1.1 Smoking Restrictions

Study participants will be allowed to use their own brand of cigarettes.

After enrollment, and between Visit 2 and Visit 3, the smokers group (group 1) will abstain from smoking for a period of 8 hours. At Visit 3, after the 8 hour smoking abstinence period, the smokers will smoke 1 single cigarette.

The subjects enrolled in the never smokers group (group 2) will not be allowed to smoke during the entire study duration.

For both groups 1 and 2, there will be no smoking restriction after the discharge at Visit 3 and during the 1 day follow-up period.

6.1.2 Dietary Restrictions

Study participants are not allowed to bring their own food or beverages to the investigational site. No food or drinks will be allowed during the conduct of the STT test except for the required glass of water (approximately 240 mL) before conducting the STT tests. Meals will be served according to the schedules provided in Section 9. Additional light snacks, fruits, and raw vegetables may be distributed to the study participants (except during STT tests). Consumption of water is allowed as desired (except during STT tests as described above).

6.2 Concomitant Medication

Prohibited medication is described under exclusion criteria 10 (Table 1, depicted below).

Table 1 - Prohibited medication.

Prohibited Medication	Restriction period (with Principal Investigator or designee discretion)
Short-acting antihistamines including intranasal antihistamines	3 days before Visit 1 until Visit 2
Long-acting antihistamines (i.e. Loratadine, Desloratadine)	7 days before Visit 1 until Visit 2
Over-the-counter cough and cold preparations or sleep aids containing antihistamines	3 days before Visit 1 until Visit 2
Leukotriene inhibitors	14 days before Visit 1 until Visit 2
Oral or intra-articular steroid	30 days before Visit 1 until Visit 2
Intranasal and inhaled corticosteroids	14 days before Visit 1 until Visit 2
Use of monoamine oxidase inhibitors	14 days before Visit 1 until Visit 2
Decongestants	48 hours before Visit until Visit 2
Cromolyn products	14 days before Visit 1 until Visit 2
Beta-adrenergic blockers (i.e. Acebutolol, Atenolol, etc.)	14 days before Visit until Visit 2
Anticholinergics	7 days before Visit 1 until Visit 2
Herbal or natural product remedies for allergy symptoms	On the day of Visit 1 until Visit 2
Short-Acting Beta Agonists	6 hours prior to spirometry (except as per protocol before spirometry)

Long-Acting Beta Agonists	3 days before Visit 1 until Visit 2
Phosphodiesterase 5 inhibitors (i.e. Sildenafil, Vardenafil, Tadalafil)	7 days before Visit 1 until Visit 2
Amiloride	3 days before Visit 1 until Visit 2
Macrolide antibiotics	7 days before Visit 1 until Visit 2
Guaifenesin	3 days before Visit 1 until Visit 2
Mucolytic agents	14 days before Visit 1 until Visit 2
Topical menthol products	14 days before Visit 1 until Visit 2
Topical nasal medication	4 weeks before Visit 1 until Visit 2
Topical ocular medication	4 weeks before Visit 1 until Visit 2
Any other medication at the Principal Investigator or designee's discretion that might interfere with the endpoints or procedures.	As per Principal Investigator or designee.

Note: drugs included in the list above cannot be used during the study, from enrollment until discharge on Visit 3. In case the subjects use any of the above mentioned drugs, that use will be recorded in the eCRF and the Principal Investigator will decide on the subject permanence on the study.

7 STUDY PROCEDURES

Personnel performing study assessments must have the appropriate training and full documentation. An overview of all study assessments is shown in the Schedule of Events (Appendix A). In this section, only the expected/planned timepoints for the various assessments are described. As not all study participants can undergo a procedure at the same time, adequate time windows are given for each study procedure and each timepoint (Section 9). Site personnel will adhere to the internal SOPs for all activities. Appropriate medical advice will be provided to the study participant in case of any medical findings requiring health care.

7.1 Informed Consent

Prior any study assessments is performed, the subject will be asked to provide his written consent to participate to the study (ICF/subject information sheet for study participation) (Section 1.3). All assessments must start after the time of ICF signature by the subject for study participation.

7.2 Clinical Assessments

Any clinically relevant medical condition detected at Visit 1 has to be documented as a concomitant disease. This also applies to clinically relevant findings in laboratory values and vital signs detected at Visit 1. Any untoward medical occurrence in a study participant detected during the study which was not present at Visit 1 must be documented as an AE. Worsening of a pre-existing condition from Visit 1 onwards will also be documented as an AE. If a clinically relevant finding is detected at Visit 1, the Principal Investigator or designee will need to check if the inclusion criterion No. 4 is still fulfilled.

The results of the clinical assessments described in this section will be recorded in the CRF.

Any abnormalities or deviations outside the normal ranges for any clinical testing (laboratory tests, etc.) can be repeated at the discretion of the Principal Investigator or designee and judged to be not clinically significant for study participation. Any abnormalities or deviations outside

the normal range for vital signs can be repeated by clinical staff and judged by the Principal Investigator or designee to be not clinically significant for study participation.

7.2.1 Demographic Data

See Appendix A - Schedule of Events for the timepoints of assessment. Demographic data (sex, age, and ethnicity) will be recorded.

7.2.2 Identification of the Current Cigarette Brand

Information on the current cigarette brand will be collected in the smoking history.

7.2.3 Questions on Smoking History/Habits and Intention to Quit Smoking.

Study participants will be asked the following questions about their smoking history and habits:

1. Have you ever smoked 100 cigarettes or more in your life) (yes/no)
   If answer is Yes, move to question 2. If answer is No, move to question 8.
2. What brand of cigarettes do you smoke (free text)
3. Are you planning to quit smoking in the next 3 months? (yes/no)
4. Have you smoked at least 20 cigarettes per day for at least the past 5 consecutive years? (yes/no)
5. How many years have you smoked cigarettes in your entire life? (numeric response, 2 digits)
   a. On average, how many cigarettes per day have you smoked during these years? (numeric response, 2 digits)
6. When you smoke cigarettes, do you mostly exhale smoke through the nose? (yes/no)
7. Have you used any of the following tobacco/nicotine containing products over the last 3 months?

	Yes	No
a. E-cigarette	☐	☐
b. Cigar	☐	☐

c. Pipe or Hookah	☐	☐
d. Snuff or chewing tobacco	☐	☐
a. Nicotine products to help you quit smoking (patch, gum, …)	☐	☐
a. Other	☐	☐

8. Have you smoked any cigarettes in the past 3 years? (yes/no)
9. Have you used any of the following tobacco/nicotine containing products over the last 3 years?

	Yes	No
a. E-cigarette	☐	☐
b. Cigar	☐	☐
c. Pipe or Hookah	☐	☐
d. Snuff or chewing tobacco	☐	☐
a. Nicotine products to help you quit smoking (patch, gum, …)	☐	☐
a. Other	☐	☐

This self-reported cigarette daily consumption will be used to assess eligibility.

7.2.4 Medical History, Concomitant Disease, Previous and Ongoing Medications

Relevant medical history will be documented at the Screening Visit. Medical history is defined as any condition that started and ended prior to the Screening Visit. Any concomitant disease will be documented at the Screening Visit. A concomitant disease is defined as any condition that started before and was going on at the time of ICF signature.

All medication taken 30 days prior to Visit 1 and all concomitant medication taken during the study will be documented in the source documentation and recorded in the CRF. Medication which was started prior to Visit 1 and which is still being taken by the study participant during the study as well as medication that is initiated after Visit 1 will be considered as a concomitant

medication. Medication initiated after Visit 1 is also referred to as concomitant medication. This applies to both prescription and over-the-counter products.

Records of medication taken include the drug name (preferably both generic and trade name), route of administration (*e.g.*, oral, intravenous), total daily dose/unit (*e.g.*, expressed in mg, mL or international units, IU), indication, the start and if applicable, the stop date (day, month and year). Any therapy changes (including changes of regimen) during the study have to be documented. Any concomitant medication that is still being taken by the study participant at the EOS will be recorded in the CRF.

7.2.5 Brief physical examination and nasal and throat exam.

See Appendix A - Schedule of Events for the timepoints of assessment. A brief physical examination (including an examination of the head and neck, chest, heart (cardiovascular) and abdomen), and a nasal and throat exam, will be performed at Screening and at study discharge or at early termination. Additional physical examinations may be performed throughout the study as judged by the Principal Investigator or designee.

7.2.6 Body Height and Weight

See Appendix A - Schedule of Events for the timepoints of assessment. Body weight and height will be measured.

Body mass index (BMI) will be calculated from the body weight and height using the following formula:

$$BMI = \frac{\text{weight in kilograms}}{\text{height in meters}^2} \quad (\text{kg/m}^2)$$

At Visit 1, BMI will be calculated from the height and weight recorded at Visit 1.

7.2.7 Vital Signs

See Appendix A - Schedule of Events for the timepoints of assessment. Systolic and diastolic blood pressure, pulse rate and respiratory rate will be measured at each visit. All measurements will be made according to the latest version of SOP titled *Vital Signs Measurement*.

7.2.8 Other Clinical Assessments

7.2.8.1 Spirometry post-bronchodilator

See Appendix A - Schedule of Events for the timepoints of assessment.

Spirometry post-bronchodilator testing is mandatory during the screening for all study participants.

Spirometry post-bronchodilator testing will be performed in accordance with the latest version of SOP titled *Spirometry*, latest version of SOP titled *Reversibility testing* and also according to the American Thoracic Society (ATS) recommendations. (Miller et al., 2005)

Bronchodilator Dosing

1. Bronchodilator dosing should be performed after baseline spirometry has been completed following latest version of SOP and also according to the ATS recommendations. (Miller et al., 2005)
2. The chosen bronchodilator will be Salbutamol and the selected dose will be 90 µg by using a metered dose inhaler (MDI). The MDI should be primed according to the manufacturer's instructions.

3. Four separate doses (one puff) of the inhaled bronchodilator will be administered by MDI in one long, slow breath to total lung capacity (TLC).
4. The doses should occur approximately 30 seconds apart using a spacer.
5. Each inhaled dose of the inhaled bronchodilator should be held by the study participant for 5-10 seconds.

10 to 15 minutes after the inhaled bronchodilator is given, spirometry maneuvers should be initiated following latest version of SOP CO-038 and also according to the ATS recommendations. (Miller et al., 2005).

The doses, the number of sprays administered and the interval period between administration of the bronchodilator and the assessment, will be recorded on dedicated log.

7.2.8.2 Drug screening and alcohol breath test

See Appendix A - Schedule of Events for the timepoints of assessment. All study participants will undergo a drug screen and an alcohol breathalyzer test on Visit 1 and Visit 2.

7.2.8.3 Exhaled carbon monoxide (eCO)

See Appendix A - Schedule of Events for the timepoints of assessment. An eCO measurement will be performed using a carbon monoxide breath monitor on Visit 1 and Visit 2. For this procedure, study participants must first inhale fully and exhale steadily into the carbon monoxide breath monitor for at least 10 seconds.

7.2.8.4 Skin prick test (SPT)

See Appendix A - Schedule of Events for the timepoints of assessment. SPT will be carried out according to applicable SOP using the Test Applicator Application and a standard panel of allergens, including seasonal allergens such as major trees, grass, ragweed and perennial allergens including cat, dog and dust mite will be tested.

7.2.8.5 Urine cotinine

See Appendix A - Schedule of Events for the timepoints of assessment. A urine cotinine screen will be performed on Visit 1 and Visit 2.

7.2.8.6 Plasma nicotine samples

See Appendix A - Schedule of Events for the timepoints of assessment.

a) Sample collection

- 1 x 7 mL of blood will be collected at each time point in pre-chilled K3EDTA vacutainers.
- Samples will be sequentially collected by direct venipuncture or catheter and processed in a timely manner as per appropriate Analytical Method Information Sheet (AMIS).
- The labels for all biological sample collection and storage containers will contain, at a minimum:
  - Subject number
  - Study number
  - Collection date
  - Scheduled collection time (study hour)

b) Sample processing

- Samples will be cooled in an ice bath or cooling device until processed by centrifuging at approximately 3000 rpm (+/- 50 rpm) at 4°C (± 4°C) for 15 minutes and then placed into an ice bath or cooling device. Samples that are disturbed during the sampling process will be re-spun under the same conditions in an attempt to obtain the maximum amount of plasma from each sample.
- The time between blood collection and placement in the centrifuge will not exceed 30 minutes (+/- 15 mins).

- Plasma will be evenly divided and transferred into duplicate 5 mL polypropylene tubes and maintained in the ice bath or cooling device.
- A minimum of 1.5 mL of plasma is required for each aliquot.
- Samples will then be stored at approximately -20°C (±10°C) until shipment to the bioanalytical laboratory.
- The time between the start of centrifugation and placement in freezer will not exceed 1 hour (+/- 15 mins).
- In cases where the volume of plasma harvested from the blood sample is not sufficient to meet the minimum volume requirement as indicated by the bioanalytical facility, the first polypropylene tube will be filled to meet the minimum volume requirement, and any remaining plasma will be transferred to the second polypropylene tube.

c) Sample shipment

- The clinical staff will inventory the samples for Group 1 (smokers) and Group 2 (never smokers) which are to be shipped to the bioanalytical laboratory. Each shipment will contain a complete set of samples for each group. The complete set of samples for Group 1 and 2 will be sent first. The second set of samples for Group 1 and 2 will not be shipped until the status of the first shipment is determined. The inventory record will accompany the plasma samples as per SOPs.
- The samples will be packed in ample dry ice within a Styrofoam container to ensure the samples will remain frozen for at least 72 hours and shipped via express delivery to the bioanalytical facility. Temperature during shipment is recommended to be monitored, using appropriate devices for the shipment. Written notification of sample shipment will be communicated to the bioanalytical facility and Sponsor. The samples will be tracked to ensure arrival in a safe and timely manner.
- The shipment will be accompanied by logs identifying the:

  study number, protocol number, number of subjects, number of samples included in the shipment and sample collection log sheets.

- Documentation noting the conditions of the samples upon arrival at the bioanalytical laboratory and whether the amount of dry ice remaining is adequate or inadequate will be returned to the clinic.
- Temperature during shipment is recommended to be monitored, using appropriate devices for the shipment.

- The samples will be shipped to:

d) Bioanalytical sample analysis

- The Nicotine plasma concentrations will be measured using validated LC/MS/MS bioanalytical method and according to the Sannova Analytical Inc's Standard Operating Procedures and FDA guidelines and applicable regulatory requirements.

7.2.8.7 Saccharin transit time test

See Appendix A - Schedule of Events for the timepoints of assessment. NMC rate will be assessed using saccharin transit time. Before performing the test, each study study participant will spend approximately 15 minutes acclimatizing to the environment of the clinic. A 1-2 mm particle of saccharin will be placed under direct vision, approximately 1 cm behind the anterior end of the inferior turbinate. The position of the study participant's head should be flexed approximately 10° by visual assessment. The study participant will be instructed not to sniff, sneeze, cough, eat or drink and instructed to remain in a seated position for the duration of the test. If possible, the study participant will also be instructed not to speak or engage in any other activities that would disrupt the evaluation. The saccharin transit time will be determined as

the elapsed time in minutes and seconds from placement of the saccharin until the time the study participant perceives a sweet taste.

In case there is no response, the test will be terminated after 60 minutes. The study participant will then have a direct application of saccharin on their tongue to ensure that they can taste the saccharin.

7.2.8.8 Nasal scraping, sical assessment and biological specimen collections for further analysis

See Appendix A - Schedule of Events for the timepoints of assessment.

Nasal scraping of the inferior turbinate of both nostrils will be performed on all 14 study participants as described in the separate document "Method establishment to obtain human nasal epithelial sample using the Rhino-probe".

For 4 study participants, nasal scraping (left nostril with method 1, right with method 2) will be performed and cell samples will be collected, processed, appropriately labeled and shipped by the study site as per the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe" to the appropriate laboratory for further histological assessment.

For the 10 remaining study participants, 5 will undergo nasal scraping using method 1, and 5 using method 2. For all 10 study participants, cell sample from one nostril will be added into one buffer and cell sample from the other nostril into a second buffer. Samples will be collected, processed, appropriately labeled and shipped by the study site as per the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe" to the appropriate laboratory for subsequent RNA quantity and quality analysis.

RNA quantity and quality will be assessed as per the Sponsor internal laboratory SOPs. RNA from the nasal sample will be extracted. Then a quantification step will occur using a Nanodrop 1000 instrument (TermoFisher scientific).

The quality will be assessed by using a Bioanlayzer 2100 (Agilent Technologies): the Bioanalyzer delivers a mark from 1 to 10 (10 is the best) to give an idea of the quality and integrity of the RNA (RIN value stands for RNA integrity Number).

7.3 Laboratory Assessments

7.3.1 Hematology, clinical chemistry, urine analysis and serology

For hematology, clinical chemistry and virology, up to 22.5 mL of blood per assessment will be needed. The following parameters will be measured:

The blood samples will be sent to LabCorp Raritan for analysis.

A labeling system will be devised such that each sample is distinguishable from all others. Each collection label will contain the following information: 1) study protocol number, 2) sample type, 3) study participant number, and 4) date and time of collection.

Table 2 - Hematology and clinical chemistry parameters

Hematology	Clinical Chemistry

• Hematocrit
• Hemoglobin
• Mean corpuscular hemoglobin (MCH)
• Mean corpuscular hemoglobin concentration (MCHC)
• Mean corpuscular volume (MCV)
• Platelet count
• Red blood cell (RBC) count
• White blood cell (WBC) count
• Differential WBC count:
  • Neutrophils
  • Basophils
  • Eosinophils
  • Lymphocytes
  • Monocytes
	• Albumin
	• Total protein
	• Alkaline phosphatase (AP)
	• Alanine aminotransferase (ALT)
	• Aspartate aminotransferase (AST)
	• Blood urea nitrogen (BUN)
	• Creatinine
	• Fasting Glucose
	• Fibrinogen
	• Gamma-glutamyl transferase (GGT)
	• Lactate dehydrogenase (LDH)
	• Potassium
	• Sodium
	• Total and direct bilirubin
	• Total cholesterol
	• Triglycerides

The following parameters will be analyzed semi-quantitatively in urine:

- pH
- Bilirubin
- Glucose
- Nitrite
- Red blood cell traces
- Protein
- Specific gravity

A test for Hepatitis B surface antigen, hepatitis C virus and human immunodeficiency virus (anti-HIV1/2) will be done at Visit 1.

7.4 Volume of blood sample

After enrollment, a total of four (4) blood samples (28 mL total volume) will be collected for nicotine analysis.

A total of 50.5 mL of blood will be collected for each subject.

8 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Events

For the purpose of this study an AE is defined as any untoward medical occurrence that may present itself during the conduct of a study and which may or may not have a causal relationship with the study procedures. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease.

8.1.2 Serious Adverse Events

A serious adverse event (SAE) is defined as an AE that:

- Results in death.
- Is life-threatening.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Is an important medical event.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

"Life-threatening" means that the subject was at immediate risk of death from the event. It might have caused death if it had occurred in a more serious form.

8.2 Assessment of Adverse Events

The Principal Investigator or designee is responsible for obtaining, assessing and documenting all AEs during the study.

Any exacerbation/worsening of an AE or pre-existing condition shall be evaluated and recorded.

AEs will be coded at the end of the study in the latest Medical Dictionary for Regulatory Activities (MedDRA) version, preferred term, available at the time of adverse events coding.

8.2.1 Collection of Information

Any non-serious AE occurrence during the study must be documented in the subject's medical records in accordance with the Principal Investigator's normal clinical practice and on the AE page of the CRF. SAEs that occur during the study must be documented in the subject's medical record, on the AE CRF, and on the SAE form.

AEs should be collected mainly via face-to-face interview with the subject through spontaneous reporting or by the use of consistent, open, non-directive questions from the PI(s) or designee(s) (e.g., "Have you had any health problems since you were last asked? or "How have you been feeling since you were last asked?"). Information recorded will include: verbatim description of the AE/SAE, start and stop dates, seriousness, severity (intensity), action taken (e.g., whether or not the AE/SAE led to the subject's discontinuation from the study), and outcome (e.g., resolved, stabilized). Information to be recorded about a SAE should also include, whenever possible, onset and resolution dates and times, circumstances leading up to the event, clinical elements such as clinical course, specific vital signs and test results that may explain the pathophysiology of the event, as well as alternative explanations to its occurrence.

Whenever a medically meaningful diagnosis is available to comprise a set of reported signs and/or symptoms, it should be preferentially provided as the AE or SAE term, rather than the individual signs and/or symptoms. Otherwise, each one of those signs and/or symptoms should be reported separately as event terms.

The following details will be recorded for AEs:

- Description of event/symptom
- Onset date and time of event
- End date and time of event
- Severity/intensity rated as follows:

  Mild: Awareness of symptoms but easily tolerated, no disruption of normal activities.

  Moderate: Discomfort enough to cause interference with usual activity.

  Severe: Incapacitating with inability to work or do usual activity.

- Any other action taken (such as concomitant medication, non-drug therapy, both, or none)
- Outcome of AE noted as follows:
  - Fatal
  - Not recovered/not resolved
  - Recovered/resolved
  - Recovered/resolved with sequelae
  - Recovering/resolving
  - Unknown

8.2.2 Period of Collection

Starting from the time when consent was obtained, the PI or designee will record all adverse events observed, queried, or spontaneously reported by the study participants. An adverse event query will be performed as scheduled as per Principal Investigator or designee's direction at the screening visit and throughout the confinement period and prior to being released from

confinement. Study participants will be asked non-leading questions such as "How do you feel now?", "How have you felt since last asked?", or "Have you taken any medication since last asked?" If the presence of any symptom(s), adverse event(s), and/or concomitant medication is recorded, the clinical staff may advise the study participants to remain at the clinic site for safety reasons until the Principal Investigator or designee decides it is safe for the study participants to leave. If the study participant decides to leave despite the advice of the Principal Investigator or designee, he/she will be asked to sign a waiver.

Any non-serious AE that is ongoing during the Safety Follow-Up Period will be actively followed-up by the Principal Investigator or designee during that period until it has been resolved, stabilized (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

At the end of the Safety Follow-Up Period, all ongoing non-serious AEs will be documented as "ongoing" and no follow-up information will be sought for on them anymore by the Principal Investigator or designee. At that point, the Investigator will assess whether the subject should be referred to his/her General Practicioner to have their ongoing AEs addressed accordingly.

All SAEs will be followed up by the Investigator or designee, despite their continuation after the end of the Safety Follow-Up Period, until their resolution, stabilization (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

8.2.3 Intensity of Adverse Event

Mild: Awareness of symptoms but easily tolerated, no disruption of normal activities.

Moderate: Discomfort enough to cause interference with usual activity.

Severe: Incapacitating with inability to work or do usual activity.

Change in severity (intensity) needs to be documented.

8.2.4 Relationship to Study Procedures

This study will not involve administration of any investigational product and therefore, the Principal Investigator or designee will make a determination of the relationship of the AE to study procedures (not related, related) according to the following guidelines:

Related:	This relationship suggests a probable temporal sequence of the event with study procedure(s) exists. Based upon the Principal Investigator, the association of the event with the study procedure(s) seems probable and the event is unlikely to be attributed to concurrent diseases or other drugs or chemicals.
	This relationship suggests a reasonable temporal sequence of the event with study procedure(s) exists. Based upon the Principal Investigator's clinical experience, the association of the event seems possible.
Not Related:	The event has been judged by the Investigator to have no relationship to study procedure(s).

8.3 Reporting of Serious Adverse Events

Any SAE observed during the period of collection by any of the parties involved in this study (including the Investigational site personnel) must be reported by that party within 24 hours of first awareness to the sponsor representative. The Sponsor representative will be notified in writing (SAE report form) within 24 hours of when a SAE is first recognized or reported by contacting:

Sponsor representative contacts:

Company: United BioSource Corporation (UBC) Pharmacovigilance
Fax: [redacted]
Email: [redacted]

Subsequently, a summary of the SAE will be sent to the sponsor representative within three working days of the original notification.

The IRB will be notified in writing (*e.g.*, facsimile) within 24 hours (1 working day) of when a reportable SAE is first recognized or reported. In addition, a copy of the written confirmation or summary of the SAE, as submitted to the sponsor representative, will also be submitted to the IRB within 3 working days of when the reportable SAE is first recognized or reported. The IRB's serious and unexpected adverse experience submission form will be completed and submitted with the copy of the written confirmation or summary of the SAE.

As further information regarding an already reported SAE becomes available to any of the parties involved in this study, such follow-up information should be reported to the sponsor representative and if applicable to the IRB, according to the same timelines described above.

8.4 Adverse Events Leading to Discontinuation

Study participants will be advised they are free to withdraw from the study at any time. Over the course of the study, the Investigator and/or the Sponsor may discontinue participation of any study participant from the study in the case of unnecessary risk, adverse events, or noncompliance. When a study participant withdraws or is discontinued from the study, all safety data normally required at the end of the study will be obtained, if possible.

Subjects who are discontinued from the study because of an AE will undergo the early termination procedures as soon as practical after the day of discontinuation and will enter the period of safety follow-up. Any AEs or SAEs that are ongoing at the end of the Safety Follow-up Period will be managed as described in section 8.2.2.

If, in the opinion of the Investigator, Sponsor, or the IRB, the incidence and severity of AE(s) outweighs the benefit of continuing the study, the study may be terminated. In the event this course of action is to be pursued, the Investigator will make every attempt to communicate

with the Sponsor prior to the decision to develop a complete plan of action and to assess outcomes.

8.5 Reporting of Other Events Critical to Safety Evaluations

8.5.1 Abnormal Results of Laboratory Tests

Any clinical safety laboratory test result that is outside of the normal reference range will be reviewed by the Investigator and assessed for clinical significance according to its severity. The severity of abnormal laboratory test result must be assessed using CTCAE 4.03 grading scales. Whenever that grading scheme is not available for the laboratory result of concern, the Investigator should assess the severity and the clinical significance of that result using his/her medical judgment.

Abnormal laboratory test results detected **at the screening visit** whose CTCAE grades are 2 or higher or still are CTCAE grade 1 and deemed clinically significant are usually concomitant disease or a manifestation of one and must be recorded accordingly. However, in some instances, they may be assessed as AEs (and therefore must be handled according to the directions in section 8.2) or as manifestations of already reported AEs. This decision will require a careful assessment of the abnormal result within the clinical context on a case-by-case basis and will depend on the Investigator's medical judgment.

Abnormal laboratory test results detected **after the screening visit** whose CTCAE grades are 2 or higher or still are CTCAE grade 1 and deemed clinically significant must be either recorded as AEs (and handled according to the directions in section 8.2) or linked to a concomitant disease or still to an already reported AE.

The principles for assessing and reporting abnormal laboratory test results, emerging after the screening visit, using CTCAE 4.03 grading scales are set up in Table 2:

Table 3 Principles for assessing and reporting abnormal laboratory test results

Grading	Clinically significant?	Is it a grade increase from previous results in study?§	Report?

Grade 1	No	Not applicable	No
Grade 1	Yes	No	No*
Grade 1	Yes	Yes	Yes, as AE or linked to an already reported AE
Grade 2 or higher	No/Yes	No	No*
Grade 2 or higher	No/Yes	Yes	Yes, as AE or linked to an already reported AE

* in this situation, this abnormal lab test result is either a manifestation of a concomitant disease or of an already reported AE.

§ grade increase in this context means the value is higher than the one from the screening visit.

Any other abnormal clinical laboratory result (including those that are not part of the core safety assessments) can, at the discretion of the Investigator, be reviewed and assessed. Even if they do not meet the criteria of the CTCAE grading scheme, the Investigator may consider them to be of clinical significance and, if they are, must report them as AEs.

In general, laboratory values will be recorded as “increased <lab parameter>” or “decreased <lab parameter>” to ensure consistency of recording/coding.

9 STUDY ACTIVITIES

9.1 Screening Visit (Day -30 to -1)

At Visit 1 (Screening Visit), study participants will undergo the procedures outlined in Appendix A to check eligibility criteria, including providing informed consent, undergoing a brief physical examination, nasal and throat exam, vital signs, skin prick test (SPT), spirometry testing, STT test, exhaled carbon monoxide (eCO) measurements, urine cotinine assessment, medical and smoking history and blood and urine collection for medical lab exams.

Following Visit 1, study participants who pass all of the inclusion criteria and do not present any of the exclusion criteria will be asked to return to the clinic for Visit 2.

9.2 Confinement Period (Day 1 to Day 2)

During Visit 2, selected inclusion and exclusion criteria will be reassessed to ensure study participants are still eligible for enrollment (Appendix B – Inclusion and Exclusion Criteria Assessment). All study participants will have baseline assessments performed (Appendix A), including STT test.

Study participants will be confined in the clinic from the beginning of Visit 2 until discharge at Visit 3. During this confinement period study participants will abstain from drinking any alcohol and any drinks containing caffeine. Standard meals will be served. Drug restrictions applicable to study participant exclusion will also be maintained until the end of the study.

No cigarettes are to be smoked from midnight (± 1 hour) between Visit 2 and Visit 3 until 8 am on the day of Visit 3. On the day of Visit 3, at least 8 hours after last cigarette, CC smokers, who want to smoke, will have a cigarette at 8 am±30 minutes and then have the STT test performed immediately after smoking (5-10 minutes post T_0). T_0 will be defined as the start of cigarette smoking for smokers. T_0 should be the same for all smokers within an acceptable time window of 1 hour. Never smokers will also have the STT test performed in the morning at 8 am ± 30 minutes (designated $T_0 \pm 30$ minutes). All study participants (smokers and never smokers) will then have the STT test performed at 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0, ensuring at least approximately 4 hours between the beginning of each STT test. The

participant will be asked to confirm he/she can no longer feel the sweet taste. Study participants will drink a glass of water (approximately 240 mL) before STT tests are performed.

Samples for plasma nicotine assessment will be collected at approximately the same time points that STT test is performed at T_0, 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post T_0.

Nasal scraping of the inferior turbinate of both nostrils will be performed on all 14 study participants at the end of Visit 3, after all other clinic assessments, following the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe".

Full procedure will be described in the "Method establishment to obtain human nasal epithelial sample using the Rhino-probe". Upon conclusion of all clinic assessments, at the time of Discharge on Visit 3, the Principal Investigator or designee will perform a brief physical examination on the study participant.

9.3 Safety Follow-Up Period

From the Discharge on Visit 3, the study participant will enter a 1-day Safety Follow-Up Period during which spontaneously reported new AEs/serious AE (SAE)s will be recorded, and the active follow-up of ongoing AEs/SAEs will be done by the site with a phone call to the study participant.

Any non-serious AE that is ongoing during the Safety Follow-Up Period will be actively followed-up by the Principal Investigator or designee during that period until it has been resolved, stabilized (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

At the end of the Safety Follow-Up Period, all ongoing non-serious AEs will be documented as "ongoing" and no follow-up information will be sought for on them anymore by the Principal Investigator or designee. At that point, the Investigator will assess whether the subject should be referred to his/her General Practicioner to have their ongoing AEs addressed accordingly.

All SAEs will be followed up by the Investigator or designee, despite their continuation after the end of the Safety Follow-Up Period, until their resolution, stabilization (i.e., no worsening of the condition), or an acceptable explanation has been found (e.g., a chronic condition).

The end of the study for a study participant is defined as the date of Discharge on Visit 3 plus one day of Safety Follow-Up Period or the date of early termination of the study participant plus one day of Safety Follow-Up Period.

The end of the entire study is defined as the end of the Safety Follow-Up Period of the last study participant.

9.4 Early Termination Procedures

If a study participant is discontinued from the study prematurely the following assessments will be performed (unless the subject refuses to do so):

- Adverse event recording
- Hematology
- Clinical chemistry
- Urine analysis
- Vital signs
- Brief physical examination.

10 QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Monitoring

The CRO (Clinical Research Organization) will designate a "Monitor" who will be responsible for the monitoring of the study. Monitoring will be performed according to CRO's SOPs and as per the agreed Monitoring Plan with the Sponsor.

The Monitor will be responsible for monitoring the study to assess compliance with the protocol, adherence to regulatory requirements, the protection of the rights and well-being of the study participants and the accuracy and completeness of reported study data recorded on the source documentation.

The Principal Investigator shall access source data for the Monitor in order that entries in the CRFs may be verified. The Principal Investigator, as part of his/her responsibilities, is expected to ensure that the study adheres to GCP requirements.

10.2 Training of Collaborators

A pre-study meeting will be conducted before site initiation. The Sponsor or its authorized representative will discuss the requirements of the Clinical Study Protocol and related documents and will also provide training to the relevant systems and other study-specific procedures. The activities of this meeting will be described in the monitoring plan.

Site staff will follow all applicable SOPs for which they will have receive proper training and comply with all applicable requirements and regulations.

10.3 Audits and Inspections

Inflamax Research, Inc. will implement and maintain quality control (QC) procedures to ensure that the study is conducted, and that the data are generated, documented, and reported

in compliance with the protocol, ICH-GCP, Inflamax Research SOPs, and applicable regulatory documents.

The Sponsor may conduct monitoring and/or audit visits at Inflamax Research, Inc. to verify adherence to the study protocol, the protection of the rights and well-being of the study participants and the accuracy and completeness of reported study data recorded on the source documentation.

11 DATA MANAGEMENT ACTIVITIES

All Data Management activities will be described in detail in the Data Management Plan (DMP) and documents specified therein. The electronic systems used to collect subject data will be Food and Drug Administration (FDA) 21 Code of Federal Regulation (CFR) Part 11 compliant.

11.1 Data Capture

11.1.1 Case Report Forms and Study Records

With the exception of electronic source data, all results from the clinical assessments will be recorded in the source data file by the Principal Investigator or designee, and then captured in the electronic CRFs at the study site. Trained study personnel will be responsible for capturing the data from the observations, tests and assessments, specified in the protocol, in the source documents and transferring the data to the eCRF according to the eCRF Completion Guidelines.

The PI has the ultimate responsibility for the collection and reporting of all data related to the clinical study and ensuring that the data are accurate, authentic/original, legible, timely (contemporaneous), enduring and available when required. The eCRF must be signed by the PI to attest that the data contained in the eCRF are true and accurate. Any corrections made to source documents must be recorded, without obscuring the original values, and must be accompanied by the date of change, reason for change and identification of the person making the change. Any change to the eCRF data will be recorded in the system's audit trail. The eCRF data will be verified against the source documents at the study site by the Monitor as described in the monitoring plan. Instances of missing or unclear data will be discussed with the PI or delegate for resolution. All questionnaires will be administered to the study participant in their local language.

11.1.2 Protocol Deviations

Protocol deviations are defined as deviations from the study procedures as defined in this document, including but not limited to, as any violation of inclusion/exclusion criteria, assessments not performed or performed outside the scheduled time windows, or use of drugs that are known to affect study endpoints.

All protocol deviations will be entered into the electronic data capture system (EDC) or other approved format.

Information from the source documents will represent the primary source of protocol deviations. Information following site monitoring and other manual reviews will be documented in the site visit reports, follow-up letters, audit documentation, or other manual review and will be recorded and tracked in the EDC or other approved format. Telecommunications and other verbal communications regarding deviations will be considered and handled as important communication, documented and tracked as Protocol Deviations, as necessary.

The overall procedure for managing protocol deviations is described in the SOPs and/or agreed upon procedure of the clinical site(s). All deviations will be reviewed periodically, as determined at study start, to identify trends to improve monitoring and/or potential impact on the statistical analysis.

11.2 Data Handling

All study data will be managed by the Data Management Team at the CRO. The Data Management Team at the CRO will prepare a Data Management Plan (DMP). This document will describe, in details, the Data Management-related procedures and processes. The overall procedures for quality assurance of clinical study data are described in the SOPs of the CRO Data Management Team and/or Data Management Plan (DMP).

Data of all study participants enrolled, including screening failures and AE during the study (from the time of Informed Consent to the end of the safety follow-up period) will be captured in the source documents. All enrolled subjects will be entered in the study database (eCRF).

All data collected during the study is declared property of the Sponsor, irrespective of the location of the database and the Data Management CRO.

11.2.1 Database Lock

The database will be soft-locked after all source data verification (SDV) has been completed,all SDV queries are resolved, all outstanding Data Management issues have been resolved and all validation, quality review and cleaning activities are complete.

After data review by the Sponsor, resolution of all raised queries and QC of the changed data, the database or selected data thereof will be declared locked upon Sponsor approval, as applicable.

Any changes to the database after that time can only be made by written agreement between the Sponsor and the Data Management and Statistical Team at the CRO. Any of those changes must be documented in the database log file.

After study completion, the study database will be transferred to the Sponsor in the format specified in the Data Management Plan in the Clinical Data Interchange Standards Consortium Study Data Tabulation Model Data Structure Specifications.

11.3 Coding

Adverse Events will be coded in the Medical Dictionary for Regulated Activities (MedDRA) and prior and concomitant medications will be coded in the World Health Organization (WHO) Drug dictionary as described in the study specific Data Management Plan (DMP).

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

Full details of the statistical analysis will be given in a Statistical Analysis Plan (SAP). Any changes to the planned statistical methods will be documented in the Clinical Study Report. The statistical evaluation will be performed using SAS®, version 9.2 or later.

STT for each time point will be summarized by smoker and never smoker group using the descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

The plasma nicotine levels for each time point will be summarized by smoker and never smoker group using the descriptive statistics mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).. The relationship between plasma nicotine levels and STT at each time point will be evaluated by spearman correlation as well as graphical presentation of these endpoints by time point and smoker and never smoker group.

An overall summary of AEs will be presented by smoker and never smoker group showing the number of events and percent of study participants who experienced AEs, SAEs, severe AEs and AEs leading to study discontinuation.

RNA concentration will be summarized by method using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI)..

RNA RIN will be summarized by method using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI).

A summary of AEs by System Organ Class (SOC) and Preferred Term (PT) by smoker and never smoker group will be presented.

Vital signs will be summarized by smoker and never smoker group using descriptive statistics: mean, minimum, maximum, median, quartiles, standard deviation (SD) and 95% confidence interval (CI)..

12.1.1 Handling of Missing Values and of Values outside the Detection Limits

Missing STT values for a time point will be treated as missing. When STT detection takes longer than 1 hour, the STT will be considered as 1 hour.

Regarding plasma nicotine samples:

- Missing sampling time: if the time of collection for a sample is unknown, that individual data point will be treated as missing data for descriptive statistics.
- Missing concentration data: missing concentration data will be treated as missing and replaced with a "." in the concentration dataset. No values will be imputed.
- Concentration data below lower limit of quantification (LLOQ): in the calculation of descriptive statistics for concentration data at each sampling time point, all LLOQ values will be treated as zero for time zero and as 1/2 LLOQ for the remaining time

12.1.2 Significance Level for Inferential Analysis

This study has no formal pre-specified hypotheses associated with the study objectives. However, 95% CI will accompany all effect estimates.

12.2 Determination of Sample Size and Power Consideration

This is an exploratory study. This study will include 14 study participants: 7 cigarette smokers and 7 never smokers. No formal powering has occurred. However, with an expected standard deviation of 2.5 minutes, a difference in STT of 3.75 minutes can be detected.

12.3 Analysis Populations

12.3.1 Full Analysis Set (FAS)

All study participants who have at least one evaluable STT test at Visit 3 will be included in FAS population.

12.3.2 Per Protocol (PP) Population

All study participants in Full Analysis Set who have no major protocol deviation will be included in the PP population.

12.3.3 Safety Population

All enrolled subjects will be included in safety population.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Investigator's and Study Administrative Structure

13.1.1 Investigator

Investigator:	Dr Frank Lee, MD Tel: +1 [redacted] Fax.: +1 [redacted] E-mail: [redacted]

13.1.2 Sponsor

Sponsor:	Philip Morris Product S.A. PMI Research & Development Quai Jeanrenaud 5 2000 Neuchâtel, Switzerland Tel: +41 (58) 242 2111 Fax: +41 (58) 242 2811

13.1.3 Bioanalytical Laboratory

Bioanalytical Lab	[redacted]

	Tel: +[redacted] Fax: +[redacted]

13.1.4 Other Responsibilities

Any SAEs will be handled by:

Company: United BioSource Corporation (UBC) Safety

Address: [redacted]

Phone: [redacted]

Fax: [redacted]

Email: [redacted]

13.2 Subject Confidentiality

Privacy and confidentiality will be maintained and governed by company procedures in compliance with the applicable federal and/or state laws as well as the Health Insurance Portability and Accountability Act (HIPAA), as applicable. Personal and medical information will not be released unless required by law.

Any documents that allow full identification of the subject (*e.g.*, the subject's signed Study Information Sheet and ICF) must be maintained in confidence by the Principal Investigator or designee. If any document relating to this study shows a subject's name or any other details relating to an identifiable person (*e.g.*, address, social security number, medical chart number, etc.), the name or other identifiable details must be obscured before a copy of that document is supplied to the Sponsor or the Sponsor's authorized representative.

13.3 Informed Consent

In accordance with 21 CFR 50, the informed consent process shall be documented by the use of a written ICF approved by the IRB. The ICF will be signed by the subject prior to any protocol-specific procedures being performed.

The Principal Investigator will explain the purpose of the study. All procedures and risks must be explained to all potential study participants in a form understandable to them and each individual must be provided with the opportunity to ask questions. The potential study participants will be informed that participation is voluntary and that they can withdraw from the study at any time.

The ICF will contain all of the elements required by ICH guidelines for GCP and any additional elements required by local regulations.

A copy of the signed ICF and any other written information will be provided to the study participant prior to study participation. The original signed ICFs will be maintained at study site.

13.4 Access to Source Documentation

Study participants will be informed that, during the course of the clinical study, the Sponsor, any authorized representatives of the Sponsor, IRB or regulatory authorities may inspect their medical records to verify the information collected and ensure that all personal information made available for inspection is handled in the strictest confidence and in accordance with national and local data protection and privacy legislation.

The Principal Investigator and all study site staff involved with the study must permit direct access to source data/documents for study related monitoring, audits, IRB review and regulatory inspection(s).

13.5 Record Retention

The Principal Investigator will maintain drug records, source documents, and signed study participant consent documents for at least 25 years unless instructed in writing by the Sponsor that records may be forwarded to the Sponsor. In accordance with Federal Regulations and ICH-GCP guidelines, these records will be available for inspection and copying if requested by a properly authorized regulatory agent.

13.6 Clinical Study Report (CSR)

A CSR for this study will be prepared regardless of whether the study is completed or prematurely terminated.

The CSR will be written based on standards of the ICH Guideline for the Structure and Content of Clinical Study Reports. In certain circumstances, an abbreviated CSR may be acceptable. Submission of the CSR to the IRB will be complied with as requested by local requirements.

The results of the additional variables for analysis, including the histological assessment of the nasal epithelium, will be presented in reports separate from the study CSR.

13.7 Financial Disclosure

Principal Investigators are required to provide financial disclosure information to the Sponsor. In addition, the Principal Investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for one year following the completion of the study.

13.8 Publication and Disclosure Policy

This document contains information that is confidential and proprietary to the Sponsor. This information is being provided solely for the purpose of evaluation and/or conducting this clinical study for the Sponsor. Disclosure of the content of this document is allowed only to qualified study staff personnel (all individuals who are qualified by education, training and experience to perform his or her respective task(s) as assigned and delegated by the Principal Investigator), IRB, or duly authorized representatives of regulatory agencies for this purpose under the condition that confidentiality is maintained. The content of this document may not be used in any other clinical study, disclosed to any other person or entity without the prior written permission of the Sponsor. The foregoing shall not apply to disclosure required by any regulations; however, prompt notice will be given to the Sponsor prior to any such disclosure.

The Sponsor plans to disclose details of the study protocol on a web-based, publicly available, clinical trial register database (*e.g.*, ClinicalTrials.gov).

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World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 64th WMA General Assembly, Fortaleza, Brazil, October 2013

15 APPENDICES

Appendix A - Schedule of Events

	Visit 1	Visit 2	Visit 3	Follow-Up
Study day	D-30 to D-1	D1	D2	D3
Informed consent	1			
Review study procedures	1	1	1	
Assessment/review of inclusion/exclusion criteria	1	1		
Demographics	1			
Body height, weight and derived BMI	1			
Medical history and concomitant disease	1			
Hematology, clinical chemistry, urine analysis	1		1 (at discharge)	
Smoking history	1	1		
Readiness to comply to study procedures, incl. smoking interruptions	1	1		
Prior and concomitant medication assessment	1	1	1	
Vital signs	1	1	1	
Nasal and throat exam	1	1		
Brief physical exam	1		1 (at discharge)	
Spirometry post-bronchodilator	1			
Serology for HIV, hepatitis B and C	1			
Alcohol breath test	1	1		

	Visit 1	Visit 2	Visit 3	Follow-Up
Drug screening	1	1		
Exhaled carbon monoxide	1	1		
Skin Prick Test	1			
Urine Cotinine	1	1		
Enrolled		1		
Plasma nicotine sampling			1[a]	
Saccharin transit time test	1	1	1[a]	
Nasal scraping			1[b,c]	
Discharge from Study			1[d]	
Adverse event recording	1	1	1	1

[a] At time: T_0 (post-cigarette), 4h (may be performed until 4h30 min), 8h (may be performed until 8h30 min), and 12h (may be performed until 12h30 min) post-T_0. For the never smokers equivalent time points will be used.
[b] After the last STT test and collection of sample for plasma nicotine.
[c] Collection of samples for subsequent histological assessment and evaluation of RNA quantity and quality.
[d] Upon completion of all study assessments.

Appendix B – Inclusion and Exclusion Criteria Assessment

Inclusion Criteria	Visit 1 (Screening)	Visit 2 (Enrollment)
1. Informed of the nature of the study and have agreed to and are able to read, review, and sign the informed consent form (ICF) prior to Screening. The subject must be willing to comply with the study procedures described in the informed consent. The informed consent document will be written in English, therefore the volunteer must have the ability to read and communicate in English.	X	
2. Male subject aged ≥25 to ≤45 years old.	X	
3. BMI between 18.0 kg/m^2 to 32.0 kg/m^2, inclusive.	X	
4. Judged by the Principal Investigator or designee to be in good health as documented by the medical history, physical examination, vital sign assessments, clinical laboratory assessments, and by general observations.	X	X
5. Belong to one of the following two groups: a. Non-menthol cigarette smoker (meets all of the following criteria at Visit 1 and at Visit 2): i. A positive urine cotinine test (≥200 ng/mL). ii. Smoked at least 20 cigarettes per day for at least the past 5 years. iii. eCO levels >10 parts per million (ppm).	X	X

iv. No plans to quit smoking in the next 3 months. b. Never smoker (meets all of the following criteria at Visit 1 and at Visit 2): i. Subject who has smoked less than 100 cigarettes throughout their lifetime and no cigarettes in the past 3 years. ii. A negative urine cotinine test (<200 ng/mL). iii. eCO levels ≤ 5 ppm.		
6. Completed the Screening process within 30 days prior to Visit 2.		X
7. Availability for the entire study period and willingness to comply with study procedures, including smoking interruptions, as evidenced by a signed ICF and at Visit 2.	X	X
Exclusion Criteria	**Visit 1 (Screening)**	**Visit 2 (Enrollment)**
1. As per the Principal Investigator or designee's judgment, the subject cannot participate in the study for any reason (e.g., medical, psychiatric, and/or social reason).	X	
2. Subject is legally incompetent, or physically or mentally incapable of giving consent (e.g., emergency situation, under guardianship, prisoners, or subjects who are involuntarily incarcerated).	X	

3. Presence of confounding allergies including allergic rhinitis and non-allergic rhinitis during the course of the study based on medical history and SPT.	X	X
4. Clinical significant abnormality on their nasal and throat exam, at the discretion of the Principal Investigator or designee at Visit 1 and/or at Visit 2.	X	X
5. Cigarette smoker who smoke/use any tobacco or nicotine products (other than CC), such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 months, as self-reported at Visit 1 or Visit 2.	X	X
6. Never smoker who smoke/use any tobacco or nicotine products, such as cigars, pipe, menthol cigarettes or electronic cigarettes in the previous 3 years, as self-reported at Visit 1 or Visit 2.	X	X
7. Cigarette smokers who state they will be unable to abstain from smoking for up to 24 hours.	X	X
8. Inability to taste sweet within 60 minutes in the STT test.	X	
9. Subjects who routinely use or who have used in the previous 4 weeks nasal sprays, inhalers or other nasal products, such as nasal irrigation (for example, Neti Pot) prior to Visit 1 and/or Visit 2.	X	X
10. Subjects who have taken any of the following medication without the indicated minimum washout period:	X	X

Prohibited Medication	Restriction period (with Principal Investigator or designee discretion)

Short-acting antihistamines including intranasal antihistamines	3 days before Visit 1 until Visit 2		
Long-acting antihistamines (i.e. Loratadine, Desloratadine)	7 days before Visit 1 until Visit 2		
Over-the-counter cough and cold preparations or sleep aids containing antihistamines	3 days before Visit 1 until Visit 2		
Leukotriene inhibitors	14 days before Visit 1 until Visit 2		
Oral or intra-articular steroid	30 days before Visit 1 until Visit 2		
Intranasal and inhaled corticosteroids	14 days before Visit 1 until Visit 2		
Use of monoamine oxidase inhibitors	14 days before Visit 1 until Visit 2		
Decongestants	48 hours before Visit 1until Visit 2		
Cromolyn products	14 days before Visit 1 until Visit 2		
Beta-adrenergic blockers (i.e. Acebutolol, Atenolol, etc.)	14 days before Visit 1 until Visit 2		
Anticholinergics	7 days before Visit 1 until Visit 2		
Herbal or natural product remedies for allergy symptoms	On the day of Visit 1 until Visit 2		

	Short-Acting Beta Agonists	6 hours prior to spirometry (except as per protocol before spirometry)		
	Long-Acting Beta Agonists	3 days before Visit 1 until Visit 2		
	Phosphodiesterase 5 inhibitors (i.e. Sildenafil, Vardenafil, Tadalafil)	7 days before Visit 1 until Visit 2		
	Amiloride	3 days before Visit 1 until Visit 2		
	Macrolide antibiotics	7 days before Visit 1 until Visit 2		
	Guaifenesin	3 days before Visit 1 until Visit 2		
	Mucolytic agents	14 days before Visit 1 until Visit 2		
	Topical menthol products	14 days before Visit 1 until Visit 2		
	Topical nasal medication	4 weeks before Visit 1 until Visit 2		
	Topical ocular medication	4 weeks before Visit 1 until Visit 2		
	Any other medication at the Principal Investigator or designee's discretion that might interfere with the endpoints or procedures.	As per Principal Investigator or designee.		

11. Subjects with evidence of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I or greater, and a forced expiratory volume 1 / the forced vital capacity ratio (FEV_1/FVC ratio) <0.7.	X	
12. Any condition the Principal Investigator or designee has cause to believe would interfere with the procedures for upper or lower airway function. This could include, but is not limited to, nasal/septum deviations, or nasal polyps or nasal allergies which will be identified by the Principal Investigator or designee.	X	X
13. Upper or lower respiratory diseases in the 4 weeks prior to Visit 2.	X	X
14. History of nasal or sinus surgery in the 5 years prior to Visit 2.		X
15. As per the Principal Investigator or designee's judgment, the subject has medical conditions which require or will require in the course of the study, a medical intervention (e.g., start of treatment, surgery, hospitalization) which may interfere with the study participation and/or study results.	X	
16. The subject has a positive alcohol test and/or a history of alcohol abuse that could interfere with the subject's participation in study at Visit 1 or Visit 2.	X	X
17. Positive urine drug screen at Visit 1 or Visit 2.	X	X

18. The subject has positive serology test for human immunodeficiency virus (HIV)1/2, Hepatitis B or Hepatitis C.		X
19. Subject has donated or been in receipt of whole blood or blood products within 3 months prior to Visit 1.	X	
20. Subject is a current or former employee of the tobacco industry or of their first-degree relatives (spouse, legal patner, parent, sibling, and child).	X	
21. Subject is an employee of the investigational site or any other parties involved in the study or of their first-degree relatives (spouse, legal patner, parent, sibling, and child).	X	
22. Subject has been in receipt of last dose from another clinical study within 3 months prior to Visit 1.	X	
23. Subject has been previously screened in this study.	X	