A RANDOMISED, CROSS-OVER, RELATIVE BIOAVAILABILITY STUDY OF NICOTINE DELIVERY AND NICOTINE EXTRACTION FROM ORAL TOBACCO PRODUCTS (TRADITIONAL SNUS, CONVENTIONAL CIGARETTE AND THREE ORAL TOBACCO-FREE NICOTINE DELIVERY PRODUCTS)

NCT# NCT04891406

Study Protocol - FINAL v2.0, 26AUG2020

Clinical Study Protocol	
Investigational Product	ZoneX #2, ZoneX #3
Sponsor study code	IB-OND-PKZX-01
Protocol Version and Date	FINAL v2.0; 26AUG2020

A RANDOMISED, CROSS-OVER, RELATIVE BIOAVAILABILITY STUDY OF NICOTINE DELIVERY AND NICOTINE EXTRACTION FROM ORAL TOBACCO PRODUCTS (TRADITIONAL SNUS, CONVENTIONAL CIGARETTE AND THREE ORAL TOBACCO-FREE NICOTINE DELIVERY PRODUCTS)

Phase	I
Indication	Healthy volunteers
Test product and dose	ZoneX #2 (oral nicotine delivery product [OND]), 5.8 mg nicotine/pouch ZoneX #3 (OND), 10.1 mg nicotine/pouch
Comparator product and dose	Skruf Snus fresh slim white, 10.9 mg nicotine/pouch [redacted] Marlboro Gold, conventional cigarette, 0.8 mg nicotine/cigarette
Sponsor signatory	[redacted] Imperial Tobacco Ltd 121 Winterstoke Road Bristol BS3 2LL, United Kingdom
Principal Investigator	[redacted] CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden
Clinical study conduct and management	CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden

1 STUDY SYNOPSIS

Study title A randomised, cross-over, relative bioavailability study of nicotine delivery and nicotine extraction from oral tobacco products (traditional snus, conventional cigarette and three oral tobacco-free nicotine delivery products)	
Study code IB-OND-PKZX-01	
Planned study period Q3 2020 to Q4 2020	**Phase of development** I
Principal Investigator [redacted] CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden	
Study design This will be a randomised cross-over, open-label, confinement study conducted in 24 male or female snus and cigarette consumers. The study will investigate 5 different nicotine containing products in a cross-over fashion. Cross-over in design, the study will incorporate pharmacokinetics evaluation, nicotine extraction evaluation, subjective questionnaire assessments as well as safety evaluation. During the study participation, subjects will come for 2 visits to the clinic, including a 5-day confinement period and finally a follow up end-of-study telephone call within a week of product use.	
Objectives <u>Primary objective</u> To evaluate and compare the maximum plasma concentration (C_{max}) and the area under the curve at the last timepoint measured (AUC_t) of nicotine after the use of each product. <u>Secondary objectives</u> To evaluate other pharmacokinetic parameters of nicotine after the use of each product. To evaluate extracted dose of nicotine in used products. To evaluate product perception and preference by use of subjective assessments. To evaluate the tolerability and safety of each of the products used. To evaluate the total nicotine exposure by measuring cotinine following single and *ad lib* use of each of the products.	
Endpoints <u>Primary endpoints</u> • C_{max} and AUC_t	

Secondary endpoints

- AUC timepoint 0 to 90 minutes (AUC_{0-90}), AUC timepoint 0 to infinity (AUC_{inf}), time to C_{max} (T_{max}), plasma concentration at last timepoint measured (C_{last}), and terminal elimination half-life ($T_{1/2}$)
- The extracted dose of nicotine from each portion, will be calculated to evaluate the correlation between AUC and extracted dose of nicotine.
- Subjective assessment endpoints from Heaviness of smoking index (HSI), Product evaluation scale (PES), Modified cigarette evaluation questionnaire (MCEQ), Products preference scale (PPS)
- Frequency, intensity and seriousness of adverse events (AEs)
- Clinically significant changes in laboratory parameters, vital signs and ECG
- Assessment of total nicotine exposure following plasma cotinine levels (concentration and applicable calculated PK parameters) following controlled single use and *ad lib* use of IP

Number of subjects planned

Approximately 48 subjects will be screened to achieve a total of 24 randomised subjects.

Main eligibility criteria

Male and female snus and cigarette consumers, aged ≥19 years and with a body mass index (BMI) of ≥ 18.0 and ≤ 30.0 kg/m^2 will be considered for participation in the study.

Pregnant or breastfeeding women, or subjects with other conditions believed to either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study, as judged by the Investigator, will be excluded from participation.

Subject who intend to change their smoking habit, reduce or stop smoking within the next 3 months will also be excluded. Subjects wishing to make a quit attempt will be offered advice by the clinical team and provided with contact information for a nicotine cessation support service.

Methodology

Visit 1 (Screening)

Visit 1 will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and assessment of snus and cigarette consuming habits. Subjects will be provided with smoking cessation advice and contact information for a smoking cessation support service, should they request it.

Visit 2 (In-clinic treatment period, Day -1 to Day 5)

At Visit 2, subjects will be admitted to the clinic on the evening of Day -1 and will remain at the clinic until Day 5 for daily single IP use and PK, nicotine extraction, subjective questionnaire assessments and safety assessments.

On the evening of Day -1, subjects will be undertaking baseline assessments for clinical laboratory profile, vital signs and ECG. Subjects are allowed to use their own snus product until 10pm on the evening of Day -1. At 10pm, the subjects' own nicotine containing

products will be collected by a member of the clinical team and returned upon completion of Day 5.

On Day -1 the subjects will also undertake a familiarisation session of the study products and questionnaires. The clinical team will explain how the products will be used and the subjects will have the opportunity to see the product and packaging. An explanation of how the questionnaires will be administered to the subjects will be given. The familiarisation session does not include a product trial and all products used in the session will not be used in the clinical study but will be retained as demonstration samples for accountability purposes.

In the morning of Day 1, after pre-use assessments and confirmation of eligibility, the subjects will be randomised and then administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. Oral nicotine IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 2, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 3, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 4, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals

throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 5, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4 and 8 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will leave the research clinic after completing all 8 h-assessments on Day 5.

Visit 3 (End-of-Study phone call)

A follow-up telephone call (Visit 3, end-of-study) will be made on Day 7 (±1) to follow-up on adverse events.

Investigational Product (IP), dosage and mode of administration

A: ZoneX #2, oral nicotine delivery product (OND), white tobacco-free nicotine pouch, 5.8 mg nicotine/pouch
B: ZoneX #3, OND, white tobacco-free nicotine pouch, 10.1 mg nicotine/pouch
C: Skruf snus fresh slim white, 10.9 mg nicotine/pouch
D: [redacted]
E: Marlboro Gold, conventional cigarette, 0.8 mg nicotine/cigarette

Duration of IP use

Single 20-minute use of each of the 4 oral nicotine products or a single cigarette use, followed by *ad lib* use of the product assigned that day until 10pm.

Duration of each subject's involvement in the study

Subjects will participate in the study for up to 35 days, including an up to 28-day screening period.

Pharmacokinetic (PK) assessments

Blood sampling for nicotine and cotinine plasma concentration analysis

Safety assessments

AEs
Clinical laboratory profile
Vital signs (blood pressure and pulse)
ECG

Subjective measures

PES, MCEQ, PPS

Statistical methods

No formal sample size calculation has been performed for this study. The proposed sample size is considered sufficient to provide adequate information for the study objectives.

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, SD, median, minimum and maximum value. In addition, for the parameters AUC and C_{max} the geometric mean and geometric coefficient of variation (CV) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).

The primary objective will be examined by the following statistical comparisons for AUC_t and C_{max}:

- Product A versus Product B, C, D and E, respectively
- Product B versus Product C, D and E, respectively
- Product C versus Product D and E, respectively
- Product D versus Product E

Log transformed nicotine C_{max} and AUC_t estimates will be evaluated separately in a linear mixed-effects analysis of variance model with fixed effects for period, sequence, and product, and a random effect for subject. If the assumptions for this model will be inappropriate will the fixed effects period and sequence be removed from the model. The above product differences will be back-transformed to present the ratios of geometric least squares (LS) means and 95% CIs of each test product versus each other from the same model.

Study reporting

After completion of the study a clinical study report (CSR) will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or term	Explanation
AE	Adverse event
ADR	Adverse drug reaction
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration-time curve
AUC_{0-90}	AUC from 0 to 90 minutes
AUC_{inf}	AUC from 0 to infinity
AUC_t	AUC from 0 to the time of the last sampling timepoint
bpm	Beats per minute (unit for pulse measurement)
BMI	Body mass index
CC	Conventional cigarette
C_{last}	Observed plasma concentration at the last sampling timepoint
C_{max}	Maximum observed plasma concentration
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CV	Coefficient of variation
DMP	Data management plan
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
GCP	Good clinical practice
GDPR	General data protection regulation
Hb	Haemoglobin
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IEC	Independent ethics committee
IME	Important medical event
IP	Investigational product
ISF	Investigator site file
LLOQ	Lower limit of quantification
MedDRA	Medical dictionary for regulatory activities
mmHg	Millimetre mercury (unit for blood pressure measurements)
NCA	Non-compartmental analysis
NGP	Next generation products
OND	Oral nicotine delivery
PII	Personally Identifiable Information
PK	Pharmacokinetic
PPS	Per protocol analysis set
PT	Preferred term
PV	Pharmacovigilance
QC	Quality control
RBC	Red blood cell
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
SOC	System organ class
SOP	Standard operating procedures
TMF	Trial master file
T_{max}	Time of occurrence of C_{max}
$T_{½}$	Terminal elimination half-life
WBC	White blood cell
WHO	World Health Organisation
WOCBP	Women of childbearing potential

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.4.6.11.

In the case of a medical emergency, the Investigator may contact the Medical Monitor (Table 4.1-1).

Table 4.1-1 Medical emergencies contact

Name	Function in the study	Telephone number and e-mail
[redacted]	Medical Monitor	[redacted]

5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor Imperial Tobacco Ltd 121 Winterstoke Road Bristol BS3 2LL, United Kingdom	**Sponsor's Project Manager** [redacted]
Clinical conduct CTC Clinical Trial Consultants AB (CTC) Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden	**Principal Investigator** [redacted]
Study management CTC Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden	**Clinical Research Manager** [redacted] **Biostatistician** [redacted] **Medical Writer (CSP author)** [redacted] **Medical Monitor** [redacted]
Laboratory (Safety)	Clinical Microbiology Uppsala University Hospital Dag Hammarskjölds väg 38 SE-752 37 Uppsala, Sweden
Laboratory (Safety)	Clinical Chemistry and Pharmacology Uppsala University Hospital Entrance 61, 2nd level SE-751 85 Uppsala, Sweden
Laboratory (Bioanalysis)	Lablytica Life Science AB Virdings allé 18 SE-754 50 Uppsala, Sweden
Laboratory (Nicotine extraction)	Reemtsma GmbH Max-Born-Straße 4, 22761 Hamburg, Germany

IP manufacturing, packaging, labelling	Skruf Snus AB, Vinkelgatan 2-4 576 33 Sävsjö, Sweden
Electronic data capture (EDC) system provider:	Viedoc Technologies AB S:t Persgatan 6 SE-753 20 Uppsala, Sweden

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Smoking is a leading cause of numerous human disorders including lung cancer, chronic obstructive pulmonary disease, and atherosclerotic cardiovascular disease. Despite the well characterised health risks, smoking rates in adult populations worldwide remain at 15% to 25%. In addition to methods of helping people quit, investigations are ongoing into the health benefits of reducing exposure to toxicants in people who continue to use tobacco. This is being done through the development of new tobacco and nicotine products, often referred to as next generation products (NGP).

Tobacco-related health risks are assumed to be due to repeated and sustained exposure to a range of smoke toxicants. Smoke from conventional cigarettes (CC) is a complex and dynamic mixture of more than 5,600 identified chemical constituents, in both its particulate and vapour phases. Some of these chemicals have been identified as potential contributors to the harmful effects of cigarette smoke and can be evaluated by measuring the levels of these chemicals themselves, or their metabolites, in urine.

Nicotine is primarily responsible for the addictive properties of cigarette smoking. Nicotine is rapidly absorbed into the bloodstream during cigarette smoking, from where it is rapidly distributed causing both systemic and central effects. In the central nervous system, nicotine acts at neuronal nicotinic receptors and this interaction may underpin its effects on mood and relaxation. The pharmacokinetic (PK) profile of nicotine during cigarette smoking is a rapid rise and fall in plasma nicotine concentrations. Correspondingly, the delivery of nicotine to the brain, and the consequent pleasurable effects experienced by the smoker, are also rapid.

It is Imperial Brands' aim to increasingly transition smokers to our NGP portfolio; products that are potentially less harmful than CC. Oral Nicotine Delivery (OND) is increasingly recognized as having the potential to reduce the risk of smoking. Oral Nicotine products also offer greater optionality in parts of the world where smoking or vaping is not permitted or where smokers prefer oral nicotine. At Imperial Brands we have added to our portfolio of NGP the ZoneX Oral Nicotine product which was launched in European markets in 2019.

6.1.1 *Summary of non-clinical data*

All Imperial Brands snus and OND products (including ZoneX) undergo rigorous risk assessment by in-house professional toxicologists and external specialists to determine the suitability of ingredients and materials. As a responsible manufacturer we continuously review this approach.

In vitro assays including the Ames test, neutral red uptake (NRU) and micronucleus assays, have been performed on smokeless tobacco products to investigate the cytotoxicity and genotoxicity of the product category, as described in the literature. The pre-clinical assessments have been performed on commercially available Swedish snus brands.

The peer-reviewed research has demonstrated that extracts of smokeless tobacco products, including Swedish-style snus (such as Skruf snus), are markedly less mutagenic, clastogenic and cytotoxic than the particulate matter of cigarette smoke. It is generally reported that the genotoxicity and cytotoxicity of smokeless tobacco products is 10% or less active than cigarette smoke, when assessed in using similar testing methodology.

6.1.2 *Clinical experience*

This is the first clinical study of ZoneX products.

6.2 Study rationale

This randomised, cross-over, open-label study is designed to primarily evaluate the relative bioavailability of nicotine delivery from oral tobacco-free nicotine delivery products, traditional snus and a conventional cigarette in male and female snus and cigarette consumers.

6.3 Risk/benefit assessment

It may be considered problematic to expose research subjects to a nicotine delivery product, the properties of which are not yet fully known. However, all research subjects are required to be daily snus consumers since at least one year (with an average or above snus consumption) and dual users of cigarettes so the participants are well acquainted with, and used to, the effects of nicotine.

As the nicotine delivery profile of a product is likely to be central to its acceptability among current tobacco users, it is reasonable to conduct formal clinical studies to assess this feature in more detail.

All risks related to use of the study product or study procedures will be explained in detail to the subjects.

Pregnant women are excluded from participation.

The potential adverse effects of the study procedures are likely to be minor and/or clinically insignificant.

Subjects will remain in the research clinic for at least 8 hours after the last use of the investigational products (IP) and will be closely monitored by medical staff.

The Principal Investigator at the research clinic will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study. The medical staff at CTC have extensive experience from Phase I studies and there are adequate procedures in place to handle unexpected and expected adverse reactions in the study subjects.

Besides the risks related to the IP as described above, there may also be risks related to the medical devices used in the study e.g. indwelling venous catheters. However, these are devices that are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. Study specific evaluations and sampling procedures, like blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable.

Risk assessment with regards to the COVID-19 pandemic:
Current recommendations from the authorities will be considered on a day-to-day basis. On-going risk evaluation, assessment sessions with Sponsors, Investigators, CRO/vendor representative members to align on local restrictions, impact assessment, contingency plans and study-specific risk mitigation strategies will be made to safeguard the study conduct and the safety of the study subjects. This study is a short-term study including a healthy population. Hence, study participation is not expected to confer increased risks to the study subjects in terms of COVID-19 exposure.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective and endpoints

Table 7.1-1 Primary objective and endpoints

Primary objective	Primary endpoints	Assessment
To evaluate and compare the maximum plasma concentration (C_{max}) and the area under the curve at the last timepoint measured (AUC_t) of nicotine after the use of each product.	C_{max} and AUC_t	Pharmacokinetic sampling and analysis, (Refer to section 11.3.1)

7.2 Secondary objectives and endpoints

Table 7.2-1 Secondary objectives and endpoints

Secondary objectives	Secondary endpoints	Assessment(s)
To evaluate other pharmacokinetic parameters of nicotine after the use of each product	AUC timepoint 0 to 90 minutes (AUC_{0-90}), AUC timepoint 0 to infinity (AUC_{inf}), time to C_{max} (T_{max}), plasma concentration at last timepoint measured (C_{last}), terminal elimination half-life ($T_{1/2}$)	Pharmacokinetic sampling and analysis, (Refer to section 11.3.1)
To evaluate extracted dose of nicotine in used products	The extracted dose of nicotine from each portion to evaluate the correlation between AUC and extracted dose of nicotine.	Collection of pouches and analysis (Refer to section 11.4.2)
To evaluate product perception and preference by use of subjective assessments	Subjective smoking effects	Product evaluation scale questionnaire (PES) (Refer to section 11.4.3) Modified cigarette evaluation questionnaire (MCEQ) (Refer to section 11.4.4) Products preference scale (PPS) (Refer to section 11.4.5)
To evaluate the tolerability and safety of each of the products used	Frequency, intensity and seriousness of adverse events (AEs)	AE reporting and questioning (Refer to section 11.4.6)

	Clinically significant changes in laboratory parameters, vital signs and ECG	Blood sampling for clinical chemistry and hematology (Refer to section 11.4.7) Blood pressure and pulse (Refer to section 11.4.8) 12-lead ECG (Refer to section 11.4.9)
To evaluate the total nicotine exposure by measuring cotinine following single and *ad lib* use of each of the products.	Assessment of total nicotine exposure following plasma cotinine levels (concentration and applicable calculated PK parameters) following controlled single use and *ad lib* use of IP	Blood sampling for analysis of cotinine (Refer to section 11.4.10)

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This will be a randomised cross-over, open-label, confinement study conducted in 24 male or female snus and cigarette consumers. The study will investigate 5 different nicotine containing products in a cross-over fashion. Cross-over in design, the study will incorporate pharmacokinetics evaluation, nicotine extraction evaluation, subjective questionnaire assessments as well as safety evaluation.

During the study participation, subjects will come for 2 visits to the clinic, including a 5-day confinement period and finally a have an end-of-study telephone call within a week of product use.

Visit 1 (Screening)

Visit 1 will take place from Day -28 to Day -1 and will include an eligibility check, review of health status and assessment of snus and cigarette consuming habits, see Table 8.1-1 for details. Subjects will be provided with smoking cessation advice and contact information for a smoking cessation support service, should they request it.

Subjects will be advised of the risks of using nicotine and referred to smoking/nicotine-use cessation support to quit if that is their intention.

Visit 2 (In-clinic treatment period, Day -1 to Day 5)

At Visit 2, subjects will be admitted to the clinic on the evening of Day -1 and will remain at the clinic until Day 5 for daily single IP use and PK, nicotine extraction, subjective questionnaire assessments and safety assessments.

On the evening of Day -1, subjects will be undertaking baseline assessments for clinical laboratory profile, vital signs and ECG. Subjects are allowed to use their own snus product until 10pm on the evening of Day -1. At 10pm, the subjects' own nicotine containing products will be collected by a member of the clinical team and returned upon completion of Day 5.

On Day -1 the subjects will also undertake a familiarisation session of the study products and questionnaires. The clinical team will explain how the products will be used and the subjects will have the opportunity to see the product and packaging. An explanation of how the questionnaires will be administered to the subjects will be given. The familiarisation session does not include a product trial and all products used in the session will not be used in the clinical study but will be retained as demonstration samples for accountability purposes.

In the morning of Day 1, after pre-use assessments and confirmation of eligibility, the subjects will be randomised and then administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. Oral nicotine IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 2, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 3, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 4, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 20 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4, 8 and 12 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will be allowed to use the IP, to which they are assigned that day, *ad lib* after the 8-hour timepoint until 10 pm.

In the morning of Day 5, after pre-IP use assessments, the subjects will be administered a single pouch/single cigarette of the IP in the sequence to which they have been randomised. IP will be used for 30 minutes with standard use instructions. The cigarette will be smoked in approximately 5 minutes with puffs taken at regular intervals approximately 30 seconds apart. PK sampling will be done pre-IP use and at 2, 5, 7, 15, 20, 30, 45, 60, 90 minutes, 2, 4, 6 and 8 hours post-IP use start. Sampling for plasma cotinine levels will be taken pre-IP use and at 4 and 8 hours post-IP use start. Used pouches will be collected for analysis of nicotine extraction. Questionnaires will be administered to the subject at defined intervals throughout the day. Safety will be followed throughout the day. Subjects will leave the research clinic after completing all 8 h-assessments on Day 5.

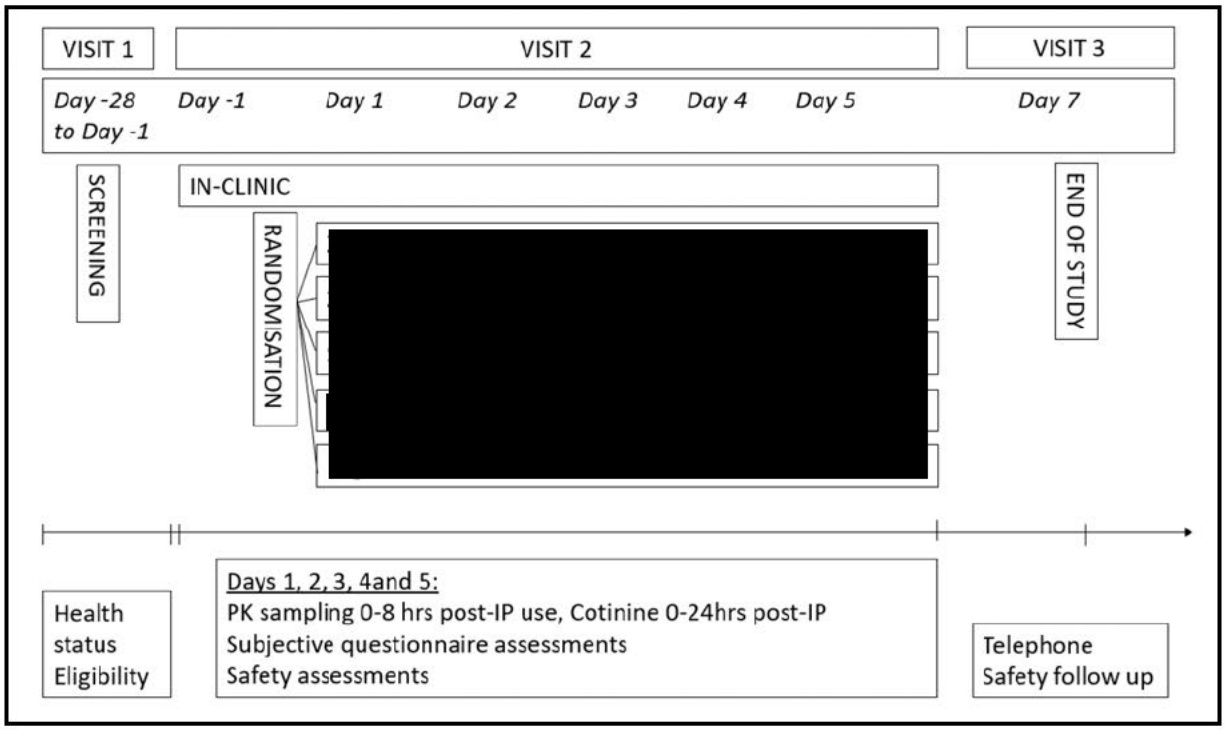
Visit 3 (End-of-Study phone call)

A follow-up telephone call (Visit 3, end-of-study) will be made on Day 7 (±1) to follow-up on adverse events.

Subjects will participate in the study for up to 35 days, including an up to 28-day screening period.

An overview of the study design is shown in Figure 8.1-1.

Figure 8.1-1 Overview of study design



The schedule of events for the study is shown overall in Table 8.1-1 and detailed for Day 1-5 in Table 8.1-2. Study assessments are described in Section 11.

Table 8.1-1 Overall schedule of events

		Screening	In-Clinic						End-of-study
Visit	**Refer to CSP section:**	**Visit 1**	**Visit 2**						**Visit 3 Phone call**
Assessment		Day -28 to Day -1	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7 (+/- 1 day)
Informed Consent	14.3	X							
Inclusion/exclusion criteria	9.4, 9.5	X	X[1]	X[1]					
Demographics	11.2.3	X							
Snus/cigarette habits	11.2.13	X							
Medical/surgical history	11.2.6	X							
HIV, hepatitis B and C	11.2.8	X							
Alcohol test	11.2.11	X	X						
Urine Drug Screen	11.2.10	X	X						
Pregnancy Test (WOCBP only)	11.2.9	X[2]	X[2]						
Weight/height (Body Mass Index)	11.2.5	X							
Physical Examination	11.2.4	X							
Clinical Laboratory Profile	11.4.7	X	X					X[3]	
Vital Signs (Blood pressure and pulse)	11.4.8	X	X	X	X	X	X	X	
12-lead ECG	11.4.9	X	X					X	
HSI	11.2.14		X						
PES	11.4.3			X	X	X	X	X	
MCEQ	11.4.4			X	X	X	X	X	
PPS	11.4.5							X	
Randomisation	9.9			X					
IP use	10.6			X	X	X	X	X	
PK blood sampling	11.3.1			X	X	X	X	X	
Cotinine sampling	11.4.10			X	X	X	X	X	
Nicotine pouch collection	11.4.2			X	X	X	X	X	
Meals[4]	9.6.1		X	X	X	X	X	X	
Baseline symptoms[5]	11.2.12	X	X	X					
Adverse events[6]	11.4.6			---------- X ----------					
Prior and concomitant medications	11.2.7	---------- X ----------							

BMI = Body mass index, WOCBP = women of childbearing potential, HIV = human immunodeficiency virus

1. Confirmation of eligibility criteria on Day-1 or Day 1
2. WOCBP only. Urine dipstick.
3. Clinical laboratory panel to include testing for SARS-CoV-2 (qPCR testing) on Day 5.
4. Meals (breakfast, lunch, snack, dinner and evening snack) will be served at the research clinic during Visit 2. On Day 1-5, lunch, snack, dinner and evening snack will be served approximately 4, 7, 9 and 11 hours, respectively, after start of each IP use. Breakfast will be served approximately 1 hour before start of each IP use.
5. Baseline symptoms will be recorded up until start of first use of IP on Day 1.
6. Adverse event will be recorded from start of first use of IP on Day 1.

Table 8.1-2 Detailed schedule of events for Day 1, Day 2, Day 3, Day 4 and Day 5

	Day 1, Day 2, Day 3, Day 4 and Day 5														
Assessment /time-point	**Pre-IP use**	**00:00**	**00:02**	**00:05**	**00:07**	**00:15**	**00:20**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**04:00**	**06:00**	**08:00**	**12:00**
Inclusion/exclusion criteria	X[1]															
Vital signs (Blood pressure and pulse)	X														X	
12-lead ECG															X[2]	
Clinical laboratory profile															X[2]	
PES	X[3]		X[3]		X[3]	X[3]	X[3]		X[3]	X[3]	X[3]	X[3]	X[3]		X[3]	
MCEQ[4]															X	
PPS															X[2]	
Randomisation	X															
IP use		---------- X ----------													*Ad lib* use until 10 pm[5]	
PK blood sampling[6]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Cotinine sampling	X												X		X	X[7]
Nicotine pouch collection								X								
Meals[8]	---------- X ----------															
Baseline symptoms[9]	X															
Adverse events[10]		---------- X ----------														
Prior and con meds	---------- X ----------															

1. Confirmation of eligibility criteria on Day-1 or Day 1.
2. Day 5 only. Clinical laboratory panel to include testing for SARS-CoV-2 on Day 5.
3. PES question 1a-g pre-use only, PES question 2a-j at timepoints 2 minutes to 4 hours, PES question 3a-y at timepoint 8 hours, PES question 4a at timepoints 30 min and 8 hours.
4. Cigarette arm only.
5. The number of units of IP used during *ad lib* use will be reported in the eCRF at the end of each treatment day.
6. PK sampling for 8 hours post-IP use on Days 1-5, respectively. Pre-use sample to be taken within 5 minutes of start of IP-use. PK sampling time windows, refer to Section 11.3.1.
7. No sample to be taken at 12 hours on Day 5.
8. Meals (breakfast, lunch, snack, dinner and evening snack) will be served at the research clinic during Visit 2. Lunch, snack, dinner and evening snack will be served approximately 4, 7, 9 and 11 hours, respectively, after start of each IP use. Breakfast will be served approximately 1 hour before start of each IP use.
9. Baseline symptoms will be recorded up until start of first use of IP on Day 1.
10. Adverse event will be recorded from start of first use of IP on Day 1.

8.2 Rationale for study design

The study will provide important safety and PK data to support the design of further studies. The time points for PK blood sampling were selected based on data obtained from previous non-clinical and clinical studies.

A crossover design was chosen to yield a more efficient comparison of treatments than a parallel study design, i.e., fewer subjects are required since each subject will serve as its own control.

A traditional snus with a nicotine strength similar to that of the investigational product was chosen as a comparator to achieve the best possible intra-subject comparison to OND products.

The purpose of the cigarette comparator is twofold; first for Imperial to begin to understand more deeply the potential harm reduction potential of next generation products, such as OND; and also to evaluate the total nicotine exposure of OND against a cigarette, in this case in a single use situation and to inform on future studies in an ad-lib usage situation.

Randomisation will be used to minimise bias in the assignment of subjects to a treatment sequence and to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups.

9 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

9.1 Recruitment

The subjects will be recruited from CTC's database of healthy volunteers and from advertising in media (including social media).

9.2 Screening and enrolment log

Investigators must keep a record of all screened subjects even if they were not subsequently included in the study. This information is necessary to verify that subjects were selected without bias. The reason for screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

A screening number will be allocated to each subject in connection to the informed consent process at the Screening visit. The screening number is generated automatically in the electronic case report Form (eCRF). The screening number will allow identification of subjects irrespective of their possible eligibility for the study.

Subjects included and randomised will be assigned a randomisation number (101, 102 etc.).

If a subject cannot perform the first planned use of IP within 28 days after screening (*i.e.*, the time interval between signing informed consent until first IP use) the subject should be rescreened before proceeding in the study.

9.3 Number of subjects

Twenty Four subjects will be included in the study. Approximately 48 subjects will be screened to achieve a total of 24 randomised subjects.

For replacements of subjects who discontinue from the study, see Section 9.8.

9.4 Inclusion criteria

For inclusion in the study, subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the study.
2. Male or female subject aged ≥19 years at the time of screening.
3. Body Mass Index (BMI) ≥ 18.0 and ≤ 30.0 kg/m^2.
4. Clinically normal medical history, physical findings, vital signs, ECG and laboratory values at the time of screening, as judged by the Investigator.
5. Dual user of snus and conventional cigarettes for ≥1 year, with a minimum weekly consumption of two or more snus cans and >5 cigarettes, and who is willing and able to use brands with nicotine content ≥1%.

9.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks of the first use of IP.
3. Any planned major surgery within the duration of the study.
4. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
5. After 10 minutes supine rest at the time of screening, any vital signs values outside the following ranges:
   - Systolic blood pressure <90 or >140 mmHg, or
   - Diastolic blood pressure <50 or >90 mmHg, or
   - Pulse <40 or >90 bpm
6. Female subjects who are pregnant or who are currently breast feeding.
7. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to nicotine.
8. Planned treatment or treatment with another IP within 1 month or investigational drug within 3 months prior to Day -1. Subjects consented and screened but not dosed in previous phase I studies are not excluded.
9. Positive screen for drugs of abuse or alcohol at screening or on admission to the research unit prior to use of the IP.
10. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator.
11. Presence or history of drug abuse, as judged by the Investigator.
12. History of, or current use of, anabolic steroids, as judged by the Investigator.
13. Excessive caffeine consumption defined by a daily intake of >5 cups of caffeine containing beverages.
14. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.
15. Subjects who intend to change their smoking habit or make a quit attempt within the next 3 months from the screening visit.
16. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

9.6 Restrictions during the study

The subjects must be willing to comply to the following restrictions during the entire study duration *i.e.*, from screening to the end-of-study visit.

9.6.1 *General restrictions*

- Subjects shall abstain from snus and all other nicotine containing products from 10 pm the evening prior to the anticipated start of each IP use on Days 1 to 5.
- Subjects shall abstain from smoking the last 24 hours before Day 1 (from approximately 8.00 am on Day -1) until the end of Visit 2 (on Day 5).
- Use of other nicotine-containing products, than defined by this study protocol, is not allowed during the study from Day-1 until the end of Visit 2.
- Subjects are not allowed to eat or drink or conduct any other mouth related procedure (e.g. tooth brushing) 30 minutes before IP use, during IP use and 30 minutes after the IP have been taken out.
- Meals and Dietary Restrictions:

  Meals will be served while the study subjects are in the research clinic. Breakfast will be served approximately 1 hour prior to IP use. Lunch will be served 4 hours after start of each IP use. Snack, dinner and evening snack will be served approximately 7, 9 and 11 hours post-IP use, respectively. Water is allowed ad libitum at the clinic except 30 minutes before IP use until one hour after IP use on Days 1-5.
- Alcohol: Consumption of alcohol is not allowed within 48 hours prior to the screening visit or Visit 2. Consumption of alcohol is not allowed during the subject's stay at the clinic during Visit 2.
- Drugs of abuse: Use of drugs of abuse is not allowed during the study (from the screening visit to the end-of-study visit). In addition to the urine drug testing described in Table 8.1-1, additional random testing can be performed at the clinic visits.
- Coffee: Consumption of up to 5 cups of coffee per day will be allowed during the study (*i.e.*, admission to the clinic on Day -1 to the end of Visit 2).
- Xanthine or taurine containing products/beverages: Energy drinks (e.g. Red bull) are not allowed during the study from admission to the clinic on Day -1 until the end of Visit 2.
- Exercise: The subjects must refrain from strenuous exercise (defined as greater than 70% of the maximal pulse rate for one hour or more) during Visit 2.
- Blood donation: The subjects must not donate blood or plasma during the study until three months after the final medical examination at Visit 2.
- Participation in other clinical studies: Study subjects are not allowed to participate in any other interventional clinical study during the study period.

9.6.2 *Prior and concomitant therapy*

Medications (prescribed or non-prescribed medication including antacids, analgesics, herbal remedies, vitamin supplements and minerals) considered necessary for the subject´s safety and wellbeing may be given at the discretion of the Investigator during the residential period. Following consultation with the Sponsor, the Investigator will determine whether or not the subject should continue in the study.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but do not fulfil all eligibility criteria and are not subsequently randomised in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

Subjects who do not meet the criteria for participation in this study may be rescreened.

Re-screening can be performed if any of the following were reasons for screening failure or non-randomisation (as judged by the Investigator):

- Practical reasons.
- Non-significant medical conditions (e.g. influenza, nasopharyngitis).
- Reserve subject in a previous group
- Plasma or blood donation outside allowed time windows.

For subjects who are re-screened, a new screening number will be assigned and a new, signed ICF will be collected.

9.8 Subject withdrawal

9.8.1 *General withdrawal criteria*

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator.

Reasons for discontinuation include:

- Subject decision
- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor
- Withdrawal of informed consent to the use of biological samples
- Pregnancy
- Death
- Meeting of an exclusion criterion during the study, which, in the opinion of the Investigator, may pose a risk for the subject

9.8.2 *Procedures for discontinuation of a subject from the study*

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If a subject withdraws consent, the Investigator must ask the subject if he/she is willing, as soon as possible, to be assessed according to the procedures scheduled for the end-of-study visit. Any ongoing AEs will be followed as described in Section 11.4.6.12.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF and final product accountability must be performed.

9.8.3 *Subject replacement*

Subjects who are prematurely withdrawn from the study for any reason may not be replaced during the course of the study.

9.9 Randomisation

On Day 1, subjects will be randomised to one of 5 treatment sequences. The five different products are:

A: ZoneX #2, OND, white tobacco-free nicotine pouch, 5.8 mg nicotine/pouch
B: ZoneX #3, OND, white tobacco-free nicotine pouch, 10.1 mg nicotine/pouch
C: Skruf snus fresh slim white, 10.9 mg nicotine/pouch
D: [redacted]
E: Marlboro Gold, conventional cigarette, 0.8 mg nicotine/cigarette

The 5 randomisation sequences are:
Sequence 1: A:B:C:D:E
Sequence 2: B:C:D:E:A
Sequence 3: C:D:E:A:B
Sequence 4: D:E:A:B:C
Sequence 5: E:A:B:C:D

A computer-generated randomisation list will be created by CTC using SAS Proc Plan, SAS Version 9.4. The randomisation list will contain subject number, sequence and treatment and will be kept by the randomiser until database lock. A copy of the randomisation list will be provided to the research clinic.

9.10 Blinding

This is an open-label study, i.e. the Investigator, study staff and subjects will know the type of IP to be received.

10 TREATMENTS

The IPs, both test- and reference products are supplied by Imperial Tobacco.

10.1 Identity of investigational products

A: ZoneX #2, OND, white tobacco-free nicotine pouch, 5.8 mg nicotine/pouch
B: ZoneX #3, OND, white tobacco-free nicotine pouch, 10.1 mg nicotine/pouch

Comparators:

C: Skruf snus fresh slim white, 10.9 mg nicotine/pouch
D: [redacted]
E: Marlboro Gold, conventional cigarette, 0.8 mg nicotine/cigarette

10.2 Identity of non-investigational products/Reference product

Not applicable.

10.3 Manufacturing, packaging and labelling

The IP, including the Skruf comparator product, is manufactured by Skruf Snus AB, Sävsjö, Sweden.

The IP will be sent direct to the clinical site from the manufacturing plant. The product will arrive in its market ready packaging (can) with the market label. Each can of product contains 24 oral nicotine pouches. The IP will be shipped to the research clinic (CTC).

The comparators [redacted] and Marlboro Gold is commercially available and will be purchased by the research clinic.

10.4 Conditions for storage

The oral nicotine products will be stored in an access-controlled storage area at CTC, at refrigerated temperature (4-8°C). The cigarette comparator will be stored in an access-controlled storage area at CTC, at room temperature (15-25°C).

The temperature is recorded continuously by an automatic temperature control system.

10.5 Preparation and accountability

IP preparation for each individual subject and day will be done by trained personnel, i.e. a site pharmacist or a registered nurse, in a dedicated room at CTC according to the randomisation list. Each individual nicotine pouch will be weighed (in grams, with 2 decimals) before administration to the study subjects. The weight of each pouch will be recorded in the eCRF.

Single units of IP for *ad lib* use will be handed out by the study nurses upon request from the subjects (see section 10.6).

CTC and the Investigator will maintain a Storage and Accountability Log as well as a Drug Dispensing Log detailing the dates and quantities of study product received, prepared for and used by each subject and IP returned or destroyed at the end of the study. No IP will be

destroyed until full accountability is completed by the monitor and the sponsor approves its destruction. Any discrepancies between prepared and returned IP must be explained and documented. Products deliberately and/or accidentally destroyed by the site or the subject must be accounted for.

10.6 Treatment administration

In the morning of Day 1 – 5, subjects will be administered a single pouch/single cigarette as randomised. See Table 10.6-1 for instructions for use of each IP.

Following completion of the single use session and after the 8 hour PK sample, the subjects will move into an ad-lib usage period during which time they will continue to use the assigned product and no other nicotine containing products, until 10pm. The number of units of IP used during *ad lib* use will be recorded in the eCRF by the end of each treatment day.

Table 10.6-1 Investigational products – Instructions for use

Investigational product	*Instructions for use*
A: ZoneX #2, OND (5.8 mg nic)	A pouch of OND is to be placed between upper lip and gum, and kept still there, for 20 minutes. Oral nicotine pouches are not intended to be chewed during the usage. The IPs should not be swallowed (swallowing saliva in IP use is allowed).
B: ZoneX #3, OND (10.1 mg nic)	A pouch of OND is to be placed between upper lip and gum, and kept still there, for 20 minutes. Oral nicotine pouches are not intended to be chewed during the usage. The IPs should not be swallowed (swallowing saliva in IP use is allowed).
C: Skruf snus Fresh Slim White (10.9 mg nic)	A pouch of Skruf is to be placed between upper lip and gum, and kept still there, for 20 minutes. Oral nicotine pouches are not intended to be chewed during the usage. The IPs should not be swallowed (swallowing saliva in IP use is allowed).
D: [redacted]	A pouch of [redacted] is to be placed between upper lip and gum, and kept still there, for 20 minutes. Oral nicotine pouches are not intended to be chewed during the usage. The IPs should not be swallowed (swallowing saliva in IP use is allowed).
E: Marlboro Gold, conventional cigarette, (0.8 mg nic)	Subjects should puff approximately every 30 seconds (and should prioritise completion of PK and Questionnaires at the 2 min timepoint). Subject should aim to complete within 10 or 11 puffs and within 5 minutes but is allows to take longer to finish the cigarette if required.

10.7 Continuation of treatment with Investigational Product

This is a study in healthy volunteers who will have no medical benefit from the treatment and thus there will be no treatment with any of the study products after end of study participation.

10.8 Treatment compliance

All IP will be administered at the research clinic under medical supervision to ensure compliance.

10.9 Return and destruction of investigational products

Any unused study product and all empty containers will be returned to the sponsor or destroyed at the site upon confirmation from the Sponsor. The Monitor will perform final IP accountability reconciliation at the study end to verify that all unused IP is adequately returned/destroyed and documented.

11 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are described in overview and in detail in the schedule of events (Table 8.1-1).

11.1 Recording of data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

It is important that PK blood sampling occurs as close as possible to scheduled time. In order to achieve this, the timing priority order at a particular time point is:

1. IP pouch collection
2. Blood samples for PK
3. Questionnaires (PES before MCEQ)
4. Standard 12-lead ECG
5. Vital signs
6. Safety laboratory samples

Time points for PK blood sampling, clinical laboratory samples, ECGs, vital signs and questionnaires are outlined in the schedule of events (Table 8.1-1 and Table 8.1-2).

Information on time windows for PK sampling, refer to Section 11.3.1.

11.2 Demographics and other baseline characteristics

11.2.1 *Informed consent*

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3.

11.2.2 *Eligibility criteria*

Eligibility criteria should be checked during screening and verified before first IP use. The criteria are specified in Sections 9.4 and 9.5.

11.2.3 *Demographic information*

The following demographic data will be recorded: gender, age, ethnicity and race.

11.2.4 *Physical examination*

A physical examination will include general appearance including skin, auscultation of lungs and heart, abdomen (liver and spleen palpation).

Any abnormalities will be specified and documented as clinically significant (CS) or not clinically significant (NCS).

11.2.5 *Weight and height*

Weight and height will be measured without shoes. BMI will be calculated, with one decimal, from the height and weight recorded.

11.2.6 *Medical/surgical history*

Medical/surgical history will be obtained by subject interview in order to verify that the eligibility criteria are met.

The medical/surgical history should include all relevant diseases and operations within 2 weeks prior to screening as judged by the Investigator.

11.2.7 *Prior and concomitant medication*

Prior medications taken within 2 weeks will be obtained by subject interview in order to verify that the eligibility criteria are met (see also Section 9.6.2).

Medications are classified as prior if the stop date was before or on the day of the first IP use (pre-IP use) and as concomitant if ongoing on the day of the first IP use, stopped after the first IP use or started after the first IP use. To distinguish between prior and concomitant medications on Day 1 (i.e. the first IP use day), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of concomitant medication from screening until the end-of-study visit must be documented appropriately in the subject's eCRF. Relevant information (*i.e.* name of medication, dose, dose form, unit, route, frequency, start and stop dates, reason for use) must be recorded. All changes in medication should be noted in the eCRF.

11.2.8 *HIV and Hepatitis B/C*

Subjects will be tested for HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, hepatitis B virus surface antigen and hepatitis C virus antibodies prior to inclusion into the study. Any positive result will exclude the subject from participating in the study.

11.2.9 *Pregnancy test*

All WOCBP will do a pregnancy test (urine dipstick) at screening and at visits specified in Table 8.1-1.

11.2.10 *Urine drug screen*

Urine will be screened for drugs of abuse at time points outlined in the schedule of events (Table 8.1-1) using the Alere™ Drug Screen Test Panel. Additional random tests can be performed during the study period.

11.2.11 *Alcohol test*

An alcohol test will be performed at time points outlined in the schedule of events (Table 8.1-1). Additional random tests can be performed during the study period.

11.2.12 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until the start of the first IP use (i.e. an event that occurs during the screening period). Such events are not AEs and will be recorded as baseline symptoms in the Medical History Log in the eCRF.

11.2.13 *Assessment of snus consumption and smoking habits*

At the screening visit, subject will be asked about their snus and smoking habits;
"Which snus product is your usual choice?"
"Which strength does the product have?" (in mg/g)
"How many portions do you use per day?"

"Which cigarette is your usual choice?"
"Which strength does the product have?"
"How many cigarettes do you smoke per day?"

Answers will be collected in the eCRF.

11.2.14 *Heaviness of smoking index*

At Day -1 subjects will answer the HSI by answering the 2 questions:
"On the days that you smoke, how soon after you wake up do you have your first cigarette?"
"How many cigarettes do you typically smoke per day?

Answers will be collected in the eCRF.

11.3 Assessments related to primary endpoints

11.3.1 *Pharmacokinetic sampling and analysis for primary endpoints*

Venous blood samples (approximately 4 mL/sample) for the determination of plasma concentrations of nicotine after use of the IP, will be collected through an indwelling venous catheter at the pre-specified time-points (Table 8.1-2).

Pre-IP use sampling may be performed within 5 minutes prior to IP use on Days 1-5, respectively.

The following time windows will apply for the PK sampling post-IP use:

± 1 minute for time-points up to 30 minutes after start of IP use.

± 2 minutes for time-points up to 90 minutes after start of IP use.

± 5 minutes for time-points up to 8 hours after start of IP use.

The actual time of collection of each sample will be recorded in the eCRF.

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged to separate plasma. The separated plasma from each blood sample will be divided into 2 aliquots in pre-labelled cryotubes and frozen at -20°C.

Plasma samples for determination of plasma concentrations of nicotine will be analysed by Lablytica AB by means of a validated using LC-MS/MS method. Samples from all evaluable subjects will be analysed.

11.4 Assessments related to secondary endpoints

11.4.1 *Pharmacokinetic sampling and analysis for secondary endpoints*

Refer to Section 11.3.1.

11.4.2 *Nicotine extraction and flavour analysis from pouches*

The individual nicotine pouch given in the morning and the first pouch for *ab lib*-use will be weighed (in grams, with 2 decimals) before administration to the study subjects. The weight of each pouch will be recorded in the eCRF.

Used pouches will be collected after 20 minutes (+/- 1 minute) of use for the determination of residual nicotine in the IPs (morning puch) and flavour analysis (*ad lib*-pouch). After the 20-minute exposure period, each pouch will be collected into a labelled poly bag, sealed and frozen. The label should have study number, day number, subject ID and product ID.

All the collected pouches will be frozen immediately at -20°C. Pouches for extraction of nicotine and flavour analysis will be analysed by Reemtsma Labs, Hamburg.

11.4.3 *Products evaluation scale*

The subject will self-assess their experience of product effect using the PES at timepoints detailed in Table 8.1-2.

The rating for each question will be done using a 100 mm VAS scale with the anchor points 0 mm (= Not at all/no urge) and 100 mm (= extremely/extreme urge) printed on paper.

The self-assessment for each question at each time point (in mm's) will be entered in the eCRF by the study personnel.

11.4.4 *Modified cigarette evaluation questionnaire*

The subject will self-assess their experience of cigarette product effect using the MCEQ (12 sub-questions) at timepoints detailed in Table 8.1-2. This questionnaire will only be administered after use of the cigarette product.

Each sub-question about how the study product made the subject feel will be assessed on paper on a 7-grade scale (1- not at all, 2- very little, 3-a little, 4-moderately, 5-a lot, 6-quite a lot, 7-extremely).

The score for each sub-question at each time point will be entered in the eCRF by the study personnel.

11.4.5 *Products preference scale*

The subject will self-assess their product preference using the PPS (2 sub-questions) at timepoints detailed in Table 8.1-2.

The sub-questions: "Please rate the 3 pouch products in order of preference." and "Please rate your use of a cigarette vs use of pouch." (1- prefer cigarette, 2- no preference, 3-prefer pouch) will be assessed at the end of Day 5.

The answer for each sub-question at each time point will be entered in the eCRF by the study personnel.

11.4.6 *Adverse events*

In this study, the IP is a nicotine product and not an investigational medicinal product. However, the procedures for monitoring, collecting and reporting of AEs will be the same as for an investigational medicinal product, as far as possible. The Principal Investigator is responsible for ensuring that all medical staff involved in the study are familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding emergencies and Phase I studies.

11.4.6.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject administered an IP and which does not necessarily have a causal relationship with this IP. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IP, whether or not considered related to the IP.

11.4.6.2 Definition of serious adverse event

An SAE is any AE which:

- results in death
- is life-threatening (this refers to a reaction in which the subject was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might had led to death if the reaction was more severe)
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (IME) (this refers to a reaction that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the other outcomes defined above)

Examples of IMEs are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency, and drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

11.4.6.3 Definition of adverse drug reaction

The term ADR is to be used whenever either the Investigator or Sponsor or designee assessed the AE as at least possibly related to the IP.

11.4.6.4 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the start of IP use until the end-of-study visit.

Any AE with start date on the day of the first IP use must be recorded with start time.

At the end-of-study visit, information on new AEs or SAEs, if any, and stop dates for ongoing events must be recorded as applicable.

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

11.4.6.5 Assessment of intensity

The intensity of each AE is to be graded by the Investigator. The intensity grades are defined as follows:

Mild	The event is usually transient and requires minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Moderate	The event is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant of permanent risk or harm to the subject.
Severe	The event interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

11.4.6.6 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the IP using the definitions below and record it the AE Log of the eCRF:

Probable	The event has a strong temporal relationship to the IP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely.
Possible	The event has a suggestive temporal relationship to the IP, and an alternative aetiology is equally or less likely.
Unlikely	The event has no temporal relationship to the IP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the IP and the event).

An AE is considered causally related to the use of the IP when the causality assessment is probable or possible.

11.4.6.7 Assessment of outcome

The Investigator must assess the outcome of an AE using the definitions below and record it on the AE Log of the eCRF:

Recovered/resolved	The subject has recovered completely, and no symptoms remain.
Recovering/resolving	The subject's condition is improving, but symptoms still remain.
Recovered/resolved with sequelae	The subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally but has some motor impairment).
Not recovered/not resolved	The subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
Fatal	
Unknown	

11.4.6.8 Reporting of action taken with study treatment

The Investigator must report the action taken with study product using the definitions below and record it on the AE Log of the eCRF:

Dose increased

Dose not changed

Dose rate reduced

Dose reduced

Drug interrupted

Drug withdrawn

Not applicable

Unknown

11.4.6.9 Collecting adverse events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

11.4.6.10 Recording adverse events

AEs must be recorded in the AE Log of the eCRF. The Investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IP; action taken, and outcome.

If the AE is serious, this must be indicated in the eCRF.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

11.4.6.11 Reporting of serious adverse events

SAE reporting should be performed by the Investigator within 24 hours of awareness via the eCRF. All available information regarding the SAE should be entered in the eCRF SAE form (i.e. term, intensity, causality, outcome, SAE criteria, action taken, narrative including rational for causality assessment) for the specific subject. By saving the event as "serious" in the eCRF and once the Investigator has signed-off of the event, an e-mail alert is automatically sent to predefined recipients to highlight that an SAE has been registered. The same information is automatically sent to sae@ctc-ab.se.

If the SAE report in the eCRF is updated and signed by the Investigator, a new e-mail alert will be sent.

In case the eCRF cannot be accessed, the SAE should be reported by manual completion of the paper SAE Form, provided in the Investigator Site File (ISF). The completed, signed and dated paper SAE Form should, within 24 hours, be scanned and e-mailed to:

Cornelia Lif-Tiberg, MD, Medical Monitor
Phone: +46 (0)73 978 94 45
E-mail: cornelia-lif-tiberg@ctc-ab.se

A copy of the paper SAE form must also be e-mailed to CTC at: sae@ctc-ab.se.

The study site should notify the site Monitor via phone or e-mail about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE should be reported electronically as well.

11.4.6.12 Treatment and follow-up of adverse events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or to the end-of-study visit, whichever comes first. At the end-of-study visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs assessed as stable by the Investigator at the end-of-study visit will not have to be followed up until resolution.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilised, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

11.4.6.13 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy of any female subjects, the use of IP must be stopped immediately, and the subject discontinued from participation in the study.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the subject was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as AEs. All outcomes of pregnancy must be reported to the Sponsor and the Principal Investigator on the pregnancy outcomes report form.

11.4.6.14 Treatment of overdose

An overdose is a dose in excess of the dose specified for each cohort in this CSP.

Over-dosing is not likely to occur in this study since all IP will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required.

An overdose should be documented as follows:

- An overdose with associated AE is recorded as the AE diagnosis/symptoms in the AE Log of the eCRF.
- An overdose without associated symptoms is only reported in the subject's medical records.

No known antidote is available.

11.4.7 *Laboratory safety assessments*

Blood samples for analysis of clinical chemistry and haematology will be collected through venepuncture or an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analysed by routine analytical methods.

Urine analysis will be performed at the research clinic using dip sticks.

The safety laboratory parameters are defined in Table 11.4-1 and will be assessed at time-points detailed in Table 8.1-1.

Any lab values outside the normal ranges will be judged as not clinically significant (NCS) or clinically significant (CS). The assessment will be recorded in the eCRF. Abnormal values assessed by the Investigator as clinically significant will be reported as AEs. If an abnormal value is associated with corresponding clinical signs or symptoms, the sign/symptom should be reported as the AE.

Subjects will be tested for ongoing SARS-CoV-2 infection as a part of the laboratory panel on Day 5 or upon development of suspected COVID-19-symptoms during the stay at the research clinic. Testing will be performed using a routine qPCR method at the local hospital laboratory. Any positive result on testing on Day 5 will exclude the subjects from the analysis of nicotine extraction and flavour analysis due to current procedures at the analysing laboratory with regards to COVID-19.

Table 11.4-1 Safety laboratory parameters

Category	**Parameter**
Clinical chemistry	Alanine aminotransferase (ALT)
	Aspartate aminotransferase (AST)
	Creatinine
	Glucose (non-fasting)
Haematology	Haematocrit
	Haemoglobin (Hb)

Category	Parameter
	Platelet count
	Red blood cell (RBC) count
	White blood cell (WBC) count
Urinalysis (dip stick)	Erythrocytes
	Glucose
	Ketones
	Leucocytes
	Nitrite
	pH
	Protein
	Specific gravity
	Urobilinogen
Pregnancy test	Urine pregnancy test (dipstick)
SARS-COV-2 qPCR testing (at Day 5 only or upon suspicion of COVID-19 symptoms)	Detection of SARS-CoV-2 virus RNA

11.4.8 *Vital signs*

Systolic and diastolic blood pressure (BP) and pulse will be measured in supine position after 10 minutes of rest.

Any vital signs outside the normal ranges will be judged as not clinically significant (NCS) or clinically significant (CS). The assessment will be recorded in the eCRF. Post-IP vital signs judged as "abnormal, clinically significant" by the Investigator will be reported as AEs.

11.4.9 *Electrocardiogram*

Single 12-lead ECG will be recorded in supine position after 10 minutes of rest using an ECG machine. HR and PR, QRS, QT and QTcF intervals will be recorded.

Safety ECGs will be reviewed and interpreted on-site by the Investigator.

Any abnormalities will be specified and documented as clinically significant or not clinically significant. Abnormal post-IP use findings assessed by the Investigator as clinically significant will be reported as AEs.

11.4.10 *Assessment of cotinine levels for total exposure*

Venous blood samples (approximately 4 mL/sample) for the determination of plasma concentrations of cotinine after use of the IP, will be collected through an indwelling venous catheter at the pre-specified time-points (Table 8.1-2).

Pre-IP use sampling may be performed within 5 minutes prior to IP use on Days 1-5, respectively.

The following time windows will apply for the cotinine sampling post-IP use:

± 5 minutes for time-points up to 24 hours after start of IP use.

The actual time of collection of each sample will be recorded in the eCRF.

Samples will be handled accoridng to the separate laboratory instructions.

11.5 Appropriateness of measurements

All methods used for safety assessments are commonly used in standard medical care and in Phase I clinical studies. Non-compartmental analysis of PK parameters is standard for Phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.3.1.

Safety laboratory samples are collected according to standard procedures.

12.2 Volume of blood

The anticipated volume of blood samples collected during the study from each subject will not exceed 450 mL (*i.e.*, less than the volume drawn during a regular blood donation).

Estimated blood volumes to be collected are presented in Table 12.2-1.

Table 12.2-1 Estimated blood volumes

Category	**Estimated number of sampling occasions**	**Estimated volume per occasion**	**Estimated total volume**
Clinical chemistry, hematology	3	20 mL	60 mL
HIV, Hepatitis B/C	1	10 mL	10 mL
PK sampling	70	4 mL	280 mL
Cotinine samples	19	4 ml	76 mL
Total:			**426 mL**

12.3 Handling, storage and destruction of laboratory samples

All biological samples will be registered in a biobank at CTC (893).

Any remains from the safety laboratory samples will be disposed of after analyses.

The samples for analyses of PK parameters will be stored at <-20°C until analysed. The samples will be disposed of after the CSR has been finalised.

All plasma samples transferred to the Sponsor's biobank will, if not used, be disposed of after 10 years.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory) keeps full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of /destroyed, if not already analysed and documented.

The Principal Investigator will ensure that:

- Subject withdrawal of informed consent is notified immediately to Sponsor.
- Biological samples from the subject, if stored at the research clinic, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the research clinic and the action is documented.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical process, system and data identification

During CSP development, the Sponsor will identify those processes, systems (facilities, computerised systems) and data that are critical to ensure human subject protection and the reliability of trial results according to applicable SOPs and International Council for Harmonisation (ICH) E6 R2.

Identified risks, *including risks associated with the COVID-19 (Coronavirus) pandemic*, will be categorised separately from the CSP.

Sponsor oversight responsibilities, such as monitoring, adverse event reporting, safety monitoring, changes in investigators and key study team staff and quality assurance activities may need to be reassessed in relation to the COVID-19 pandemic and temporary, alternative proportionate mechanisms of oversight may be required.

13.2 Quality assurance and quality control

The Sponsor is responsible for implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs with regards to management of identified risks, CSP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.

The Sponsor is responsible for securing agreements with involved subcontractors and to perform regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.

The Sponsor is responsible for implementing a risk-based validated electronic data capture system and maintain SOPs for the whole life cycle of the system.

QC should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

The Sponsor has delegated the responsibilities outlined above to CTC whilst maintaining overall study oversight.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki [1] and are consistent with ICH/GCP E6 (R2), EU Clinical Trials Directive, and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The Principal Investigator is responsible for submission of the CSP, the subject information and ICF, any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to applicable IEC for approval.

Approval must be obtained in writing from the IEC before the first subject can be recruited.

The Sponsor will provide the IEC and Principal Investigators with relevant safety information during the course of the study in accordance with local regulations and requirements.

14.3 Subject information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject adequate verbal and written information before any study specific assessments are performed.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasised that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing the ICF.

Before performing any study-related procedures the ICF must be signed and personally dated by the subject and by the Investigator. A copy of the subject information including the signed ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

14.4 Subject data protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC) and General Data protection Regulation (GDPR), the data will not identify any persons taking part in the study.

The potential study subject should be informed that by signing the ICF he/she approves that authorised representatives from Sponsor and CTC, the concerned IEC and CA have direct

access to his/her medical records for verification of clinical study procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete in accordance with the European Union Data Protection Directive (95/46/EC) and the request will be raised to the Principal Investigator.

The Investigator must file a Subject Identification List which includes sufficient information to link records, i.e. the eCRF and clinical records. This list should be preserved for possible future inspections/audits but must not be made available to the Sponsor except for monitoring or auditing purposes.

Personal data that are collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudoanonymised, i.e. personally identifiable information (PII) will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the study. After the study end, only anonymised data, i.e. aggregated data sets, can be used.

For this study, the Sponsor is the data controller of all data processed during the study (e.g. Trial Master File [TMF], study reports) and CTC AB is the data processor. Any subcontractors used in the study, are also data processors.

For data that are processed at the clinic(s) (e.g. medical records and ISF), CTC AB is the data controller.

14.5 Changes to the approved clinical study protocol

Any proposed change to the approved final CSP (including appendices) will be documented in a written and numbered clinical protocol amendment. All substantial amendments to the protocol must be approved by the appropriate IEC before implementation according to applicable regulations.

14.6 Audits and inspections

Authorised representatives of Sponsor, or an IEC may perform audits or inspections at the research clinic, including source data verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, ICH-GCP guidelines and any applicable regulatory requirements. The Investigator will contact the Sponsor immediately if contacted about an inspection at the site.

14.7 Insurance

Subjects will be covered under the Sponsor’s liability insurance policy. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering services performed by CTC.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a study initiation visit at the research site. The requirements of the CSP and related documents will be reviewed and discussed and the investigational staff will be trained in any study specific procedures and system(s) utilised.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae will be available for all staff to whom study-specific duties are delegated.

15.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities.

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor and provided separately, the responsible Monitor will periodically visit the study site at times agreed upon by the Investigator and the Monitor. *Adaptations related to the on-site monitoring plan, when it is impossible or inappropriate to follow due to the COVID-19 pandemic, may be required such as supplementation with (additional/increased) centralised monitoring and central review of data if considered possible and meaningful. Results of adjusted monitoring/review measures should be reported to the Sponsor in monitoring reports and in the CSR.* At the time of each monitoring visit, the role of the Monitor is (but not limited to) to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the CSP, applicable SOPs, guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the eCRFs and that IP accountability checks are being performed.
- verify that data in the eCRF are consistent with the clinical records (SDV) in accordance with the RBM plan.
- verify that the correct informed consent procedure has been adhered to for participating subjects.
- ensure that withdrawal of informed consent to the use of the subject's biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject.

- verify that AEs are recorded and reported in a timely manner and according to the CSP.
- raise and escalate any serious quality issues, serious GCP breach and any data privacy breach to the Sponsor.

Centralised monitoring will also be performed continuously by study team members at CTC in accordance with the RBM plan.

When the study has been completed and all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data documents

A separate Origin of Source Data List will be generated for each site before start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, that verifies the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the trial. They include laboratory notes, memoranda, material dispensing records, subject files, etc. The eCRF may constitute source data if clearly defined in the Origin of Source Data List.

The Investigator should guarantee access to source documents to the Monitor, CAs and the IECs, if required.

15.4 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study.

Agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects be enrolled.

15.5 Study timetable and end of study

The study is expected to start in Q3 2020 and to be completed by Q4 2020.

A subject is considered to have completed the study if he/she has completed all visits in the study including the last visit. The end of the study is defined as the date of the last visit of the last subject in the study.

15.6 Termination of the study

The Sponsor reserves the right to discontinue the study at any time but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the PI must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused IP and other study materials must be returned and all eCRFs completed as far as possible.

15.7 Reporting and publication

15.7.1 *Clinical study report*

After completion of the trial, a final study report will be written following the guidance in ICH Topic E3. A CSR, describing the conduct of the study, any statistical analyses performed and the results obtained, will be prepared by CTC. The report will be reviewed and approved by, as a minimum, the Principal Investigator, the Statistician and the Sponsor.

15.7.2 *Confidentiality and ownership of study data*

Any confidential information relating to the IP or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study are responsible for protecting the confidentiality of this proprietary information belonging to the Sponsor.

15.7.3 *Publication*

The results from this study may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, (as defined in ICH E6 GCP, Section 8) for 10 years after finalisation of the CSR. This includes any original source documents related to the study, the Subject Identification List (providing the sole link between named subject source records and anonymous eCRF data), the original signed ICFs and detailed records of disposition of IP.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the TMF in accordance with ICH E6 GCP, Section 8 and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the clinic and filed in the Investigator Study File for archiving for 10 years after finalisation of the CSR.

The completed eCRFs are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorised representatives of appropriate Health/Regulatory Authorities, without written permission from the Sponsor.

16 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database set-up and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the CSP.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of computerised online edit checks identifying e.g. data values that are outside the allowed range and SAS-programmed batch checks on data exports. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

Detailed information on data management will be described in a study-specific Data Management Plan (DMP).

16.1 The web-based eCRF

Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by Viedoc Technologies AB. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or at bedside (if the eCRF data constitutes source data). Source data are to be defined at the site before inclusion of the first subject (Section 15.3).

Authorised site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorised trial site personnel prior to the trial being initiated and any data being entered into the system for any study subject.

16.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that preferably the same individual who made the initial baseline determinations completes all corresponding follow-up evaluations. The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable or unknown, the Investigator or assigned clinical staff should record such information in the eCRF. The Investigator will be required to electronically sign off the clinical data. This will be performed by means of the Investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature.

16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there

are no discrepancies for critical data as described in the RBM plan. All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF.

If corrections are needed, queries will be raised within the eCRF, either as a result of built-in edit checks or manually raised by the monitor. An appropriate member of the site staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query. The monitor will either approve the answer/correction or re-issue the query.

16.4 Audit trail

All entries in the eCRF will be fully recorded in a protected audit trail. Once clinical data have been saved, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged.

16.5 External data

External data consists of data that are not recorded in the eCRF. Data may be received in electronic format or as a paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at CTC. AEs and medical/surgical history verbatim terms will be coded using the Medical Dictionary of Regulatory Activities (MedDRA; latest version available at start of coding). Prior and concomitant medications will be coded according to the World Health Organisation (WHO) Anatomic Therapeutic Chemical (ATC) classification system. All coding will be approved by Sponsor prior to database lock.

16.7 Database lock

When all data have been entered and discrepancies solved, clean file will be declared, the database will be locked and the data will be analysed.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP), which will be signed and approved prior to database lock.

Analyses of the primary and secondary endpoints will be performed by CTC.

17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, SD, median, minimum and maximum value. In addition, for the parameters AUC and C_{max} the geometric mean and coefficient of variation (CV) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).

Baseline will be defined as the visit with last data collection point prior to the first use of IP.

No imputation of missing data will be performed.

For subject questionnaires measured at each PK time point, the Bonferroni-Holm p-value adjustment will be applied.

17.2 Determination of sample size

No formal sample size calculation has been performed for this study. The proposed sample size is considered sufficient to provide adequate information for the study objectives.

Approximately 48 subjects will be screened to achieve 24 randomised subjects.

17.3 Analysis data sets

17.3.1 *Full analysis set*

The Full Analysis Set (FAS) will consist of all subjects who have been randomised and used at last one of the IPs and who has at least one post-baseline assessment of data.

17.3.2 *Safety analysis set*

The Safety Analysis Set will comprise all subjects who used at last one of the IPs.

17.3.3 *Per protocol set*

The Per Protocol Set (PPS) will consist of all subjects who have been randomised and completed the study without any major protocol deviations that are judged to compromise the analysis of the data. All protocol violations will be judged as major or minor prior to database lock.

17.3.4 *Pharmacokinetic analysis set*

The Pharmacokinetic analysis set (PKAS) will consist of all subjects who used at last one of the IPs and provided an evaluable plasma concentration profile and who have no AEs or protocol deviations judged to affect the PK analysis. Individual PK values may be excluded from the analysis as specified in the SAP. The PKAS will be used as the per protocol analysis set.

17.4 Description of study population

17.4.1 *Demographics and baseline characteristics*

Descriptive statistics for demographics, physical examinations, weight and height will be presented by sequence.

17.4.2 *Medical/surgical history and prior/concomitant medication*

Medical/surgical history will be presented by system-organ-class (SOC) and preferred term (PT). Prior/concomitant medications will be presented by ATC level 3 and 5.

Medical/surgical history and prior medications will be presented overall by descriptive statistics and listings. Concomitant medications will be presented by sequence.

All data will be listed by subject.

17.4.3 *Treatment compliance*

The number of subjects treated in sequence and their individual IP use will be listed.

17.4.4 *Heaviness of smoking index*

Scores from the individual HSI will be presented with frequency tables including number of observations and percent. The sum of the 2 questions will be presented with descriptive statistics by sequence.

17.5 Analysis of primary endpoints

17.5.1 *Analysis of pharmacokinetics*

The PK analysis will be based on the PK analysis set and performed by CTC. The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 8.1 or later (Certara, U.S.A.).

The following non-compartmental PK parameters will be determined for each IP use:

- AUC_{inf} (baseline adjusted [when possible] and non-baseline adjusted)
- AUC_t (baseline adjusted [when possible] and non-baseline adjusted)
- $AUC_{0\text{-}90}$ (baseline adjusted [when possible] and non-baseline adjusted)
- C_{max} (baseline adjusted [when possible] and non-baseline adjusted)
- C_{last} (baseline adjusted [when possible] and non-baseline adjusted)
- T_{max} (baseline adjusted [when possible] and non-baseline adjusted)

- $T_{½}$ (baseline adjusted [when possible] and non-baseline adjusted)

$Lambda_z$ (with acceptance criteria; R^2, extrapolated AUC) will be calculated to support baseline adjustment of above PK parameters.

C_{max} and T_{max} will be derived from the observed plasma concentration data. The AUC will be calculated using log-linear trapezoidal interpolation (linear - up, log – down). Calculations will be based on the actual sampling times recorded during the study. Concentrations below Lower limit of quantification (LLOQ) occurring before C_{max} will be treated as zero. Concentrations below LLOQ occurring after C_{max} will be omitted from the analysis. AUC_t, and C_{max} will be corrected for and calculated with and without nicotine baseline corrections. Where plasma nicotine concentrations are above the lower limit of quantification immediately prior to product use (-5 minutes), PK parameters will be calculated from baseline adjusted concentrations using subjects' elimination rate constant.

Summary statistics for the PK parameters will be presented by product with number of measurements, arithmetic mean, SD, CV, median, minimum, maximum, geometric mean, geometric CV%. All data will be listed by subject.

The primary objective will be examined by the following statistical comparisons for AUC_t and C_{max}:

- Product A versus Product B, C, D and E, respectively
- Product B versus Product C, D and E, respectively
- Product C versus Product D and E, respectively
- Product D versus Product E

Log transformed nicotine C_{max} and AUC_t estimates will be evaluated separately in a linear mixed-effects analysis of variance model with fixed effects for period, sequence, and product, and a random effect for subject. If the assumptions for this model will be inappropriate will the fixed effects period and sequence be removed from the model. The above product differences will be back-transformed to present the ratios of geometric least squares (LS) means and 95% CIs of each test product versus each other from the same model.

17.6 Analysis of secondary endpoints

17.6.1 *Pharmacokinetics*

Refer to section 17.5.1.

17.6.2 *In vivo extracted amount of nicotine*

The data needed for the analysis of in vivo extracted amount of nicotine are the individual data of amount of nicotine for unused reference pouches and the amount of nicotine left in the study pouches. The mean nicotine concentration of the reference pouch and weight of each individual study pouch and residual nicotine amount will be used to calculate the extracted amount. The mean of extracted amount (mg/unit) and extraction fraction (%) of nicotine for each IP pouch, will be calculated. The extracted dose of nicotine will be analysed using the signed Wilcoxon rank sum test for within subject difference (i.e. between IPs). Amount of

nicotine in reference pouches and in used pouches will be presented through descriptive statistics.

17.6.3 *Products evaluation scale*

Each sub-question, sub-category and the total score of Questions 1a-g (sub-category 1), 2a-j (sub-category 2), 3a-y (sub-category 3) and 4a will be presented using descriptive statistics at selected timepoints by product including a graph of the mean at each time point.

The total score of PES and the sub-categories will be calculated for each product and intraindividual difference for each timepoint assessed will be analysed using a Wilcoxon Signed Rank Sum test.

17.6.4 *Modified cigarette evaluation questionnaire*

Frequency (number and percentage) for each sub-question will be presented using tables.

17.6.5 *Products preference scale*

The sub-questions 1 will be presented using a frequency table with number of observations and all combinations of the subject's preference.

The sub-question 2 will be presented using a frequency table with number of observations and percent.

No statistical testing will be performed on sub-questions.

17.6.6 *Adverse events*

An overview of all AEs, including SAEs, intensity, relationship to IP, and deaths will be presented by SOC and PT.

Incidence of AEs and SAEs will be summarised by SOC and PT by sequence and overall.

All AE data will be listed by subject and include the verbatim term entered by the Investigator.

17.6.7 *Clinical laboratory analyses*

Clinical laboratory data will be summarised overall with absolute and percent change from baseline.

Abnormal, clinically significant values will be summarised separately, if considered appropriate.

All data will be listed by subject.

17.6.8 *Vital signs*

Vital signs (systolic/diastolic blood pressure and pulse) will be summarised by sequence. Data will be presented with absolute and percent change from baseline.

All data will be listed by subject.

17.6.9 *12-lead ECG*

All ECGs will be categorised as ”normal”, ”abnormal, not clinically significant”, or ”abnormal, clinically significant” (as judged by the Investigator) and summarised by sequence using frequency tables.

Changes over time will be presented using shift tables, if considered appropriate.

All data will be listed by subject.

17.6.10 *Total exposure (cotinine in plasma)*

The analysis and presentation of total nicotine exposure following plasma cotinine levels (concentration, applicable calculated PK parameters and number of *ad lib* IP units used) following controlled single use and *ad lib* use of IP will be further specified in the SAP.

18 REFERENCES

1. World Medical Association, *WMA Declaration of Helsinki – Ethical principles for medical research involving human subjects* [website], https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/, (accessed 10JUN2020)

19 SIGNATURES

19.1 Principal Investigator statement

I have read and understood this CSP and agree to conduct the study accordingly and to comply with the Investigator obligations stated in this CSP, GCP and applicable regulatory requirements.

Principal Investigator

[redacted]

CTC Clinical Trial Consultants AB
Site

19.2 Signature page (approval of the clinical study protocol)

Sponsor signatories