| **Clinical Study Protocol** | |
|---|---|
| Investigational Product | ZYN® |
| Study Code | SM 17-03 |
| Protocol Version and Date | Final 1.0, 05Oct2017 |

**STUDY TITLE**

**Nicotine pharmacokinetics and subjective effects of a single dose of a non-tobacco-based nicotine pouch (ZYN®) compared with conventional, tobacco-based Swedish snus among current, daily snus users.**

| | |
|---|---|
| **Design** | Open, randomized, five-way cross-over, single dose administration. The study will include 18 subjects. |
| **Test products and dosage** | **1=** ZYN Smooth containing 3 mg nicotine per portion<br>**2=** ZYN Smooth containing 6 mg nicotine per portion<br>**3=** ZYN Smooth containing 3 mg nicotine per portion (alternative manufacturing process)<br>**4=** ZYN Smooth containing 6 mg nicotine per portion (alternative manufacturing process) |
| **Comparator product and dosage** | **5=** Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) |
| **Sponsor signatory** | [redacted]<br>Swedish Match<br>SE-Box 17037<br>104 62 Stockholm<br>Sweden<br>Maria Skolgata 83<br>SE-118 85 Stockholm<br>Sweden<br>[redacted] |

| | |
|---|---|
| **Principal Investigator** | Jan Erik Berglund, MD, PhD<br>CTC Clinical Trial Consultants AB |
| **Clinical study conduct and management** | CTC Clinical Trial Consultants AB<br>Dag Hammarskjölds väg 13<br>SE-752 37 Uppsala, Sweden |

*The following amendments have been made to the Final Clinical Study Protocol version 1.0:*

| **Amendment No.** | **Date of Amendment** | **Revised protocol version** *(if applicable)* |
|---|---|---|
| | | |
| | | |
| | | |

## 2 STUDY SYNOPSIS

| | |
|---|---|
| **Study Title**<br><br>Nicotine pharmacokinetics and subjective effects of a single dose of a non-tobacco-based nicotine pouch (ZYN®) compared with conventional, tobacco-based Swedish snus among current, daily snus users. | |
| **Study code**<br><br>**SM 17-03** | |
| **Study period**<br><br>Estimated date of first subject enrolled:<br>Q4 2017<br>Estimated date of last subject completed:<br>Q1 2018 | |
| **Principal Investigator**<br><br>Jan Erik Berglund, MD, PhD<br>CTC Clinical Trial Consultants AB | |
| **Study design**<br><br>Open, randomized, five-way cross-over, single dose administration. | |
| **Objectives**<br><br><u>Primary objective(s)</u><br><br>To compare each subject's $AUC_{inf}$ based on plasma concentrations of nicotine after administration of one single dose of a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, to that of one single dose from a 1 g Swedish snus pouch containing 8 mg of nicotine.<br><br><u>Secondary objectives</u><br><br>1. To compare $AUC_{60min}$, $C_{max}$, $T_{max}$, AUCo-t and terminal half-life of a novel, non-tobacco-based nicotine pouch to that of a Swedish snus pouch.<br>2. To compare the estimated *in-vivo* extracted amount of nicotine from a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, respectively, with that from a 1 g Swedish snus pouch containing 8 mg of nicotine.<br>3. To compare heart rate and subjective effects ("head buzz") after study product administration (as proxy for *in vivo* nicotine uptake).<br>4. Collection of adverse events | |

**Number of subjects planned**

The study will include 18 subjects.

**Diagnosis and main eligibility criteria**

Healthy subjects aged ≥19 years who use tobacco-based snus since ≥1 year with a weekly consumption of three or more snus cans (brands with nicotine content ≤1%) or two or more cans (brands with nicotine content >1%). Subjects who are pregnant or who have a history of hypertension or any cardiovascular disease are excluded. Subjects shall be abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.

**Methodology**

Before entry to the study subjects undergo screening evaluations including medical history and pulse rate measurements before/after application of their usual brand of Swedish snus.

Subjects report to the clinic on separate days for the 5 experimental sessions. The subjects are instructed to abstain from snus or other nicotine delivery products as from the evening before and smoking 24 hours prior to all visits to the clinic. All sessions are performed during the morning hours to facilitate abstinence. The subjects should certify abstinence before each treatment is started.

The treatments are administered as single doses in a pre-determined randomized order. The subject keeps the pouch still between the upper lip and the gum for 60 minutes and are instructed not to manipulate the pouch with the tongue or lips. The subjects are instructed not to eat or drink from 30 minutes before and during application of investigational products and 30 minutes after the investigational product have been taken out. [5].

Each used pouch is collected and frozen (-20°C) pending analyses of nicotine. Unused pouches are collected and frozen (-20°C) pending analysis and serve as references in the calculations of extracted doses.

**Investigational Products, dosage and mode of administration**

**Test articles:**

**1=** ZYN Smooth containing 3 mg nicotine per portion

**2=** ZYN Smooth containing 6 mg nicotine per portion

**3=** ZYN Smooth containing 3 mg nicotine per portion (alternative manufacturing process)

**4=** ZYN Smooth containing 6 mg nicotine per portion (alternative manufacturing process)

**Reference articles:**

**5=** Swedish portion snus PSWL 1.0 g (8 mg nicotine/g)

**Duration of treatment**

The treatments are administered as single doses in a pre-determined randomized order. The subject keeps the pouch still between the upper lip and the gum for 60 minutes. Plasma concentrations of nicotine are followed over 6 hours.

**Duration of subject's involvement in the study**

Each Subject will participate in the study approximately 20-84 days.

**Efficacy assessments**

***Pharmacokinetics assessments*** **-** WinNonlin computer program (Certara Corp., USA) will be used for pharmacokinetic calculations. Nicotine plasma concentrations are determined at preset time points, before *(0), 5, 10, 15, 30 and 60* minutes, 1.5, 2, 4 and 6 hours after administration.

***Pulse rate*** – Pulse rate will be measured at the following time points: before (0), 5, 10, 15, 30 and 60 minutes after each product is administered.

***Subjective effects*** – Each subject's rating of product "strength" using a Visual Analogue Scale (VAS): (head "buzz", "head rush", "hit", feeling alert, overall "product strength"), anchored with "not at all" to "extremely". VAS scores will be obtained at the following time points before (0), 5, 10, 15, 30 and 60 minutes after each product is administered.

**Safety assessments**

Adverse Events will be collected starting with administration of the Investigational Product until the last follow-up from the first dose.

**Statistical methods**

The study will include 18 subjects. A previous study (Lunell E & Curvall M 2011) has made the calculation of sample size possible. Nicotine extraction from 1 g Swedish portion snus containing 8 mg nicotine/pouch was estimated at 2.18±0.92 mg per 1 g portion. Under the assumption of a complete dissolution and extraction of the 3 and 6 mg ZYN® products, respectively, versus the 2.18±0.92 mg nicotine, and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and alpha=0.05.

# 3 TABLE OF CONTENTS

**List of Tables**

# 4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or term | Explanation |
|---|---|
| AE | Adverse event |
| ATC | Anatomical therapeutic chemical |
| BP | Blood pressure |
| CRF | Case report form |
| CSP | Clinical study protocol |
| CSR | Clinical study report |
| CTC | Clinical Trial Consultants AB |
| CTCAE | Common terminology criteria for adverse events |
| CV | Coefficient of variation |
| DMP | Data management plan |
| DVP | Data validation plan |
| EEA | European Economic Area |
| GCP | Good clinical practice |
| h | hour |
| ICF | Informed consent form |
| ICH | International conference on harmonization |
| IEC | Independent ethics committee |
| IP | Investigational product |
| MedDRA | Medical dictionary for regulatory activities |
| min | minute |
| N | number |
| NRT | Nicotine replacement therapy |
| NSAID | Non-steroid anti-inflammatory drug |
| SAR | Serious adverse reaction |
| PPAS | Per protocol analysis set |
| PSWL | Pouched Snus White portion Large |
| PT | Preferred term |
| SADR | Serious adverse drug reaction |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |

| | |
|---|---|
| SDV | Source data verification |
| sec | Second |
| SOC | System organ class |
| SOP | Standard operating procedures |
| SUSAR | Suspected unexpected serious adverse reaction |
| TEAE | Treatment emergent adverse event |
| VAS | Visual Analogue Scale |
| WHO | World Health Organization |

# 5 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

## 5.1 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 included in Section 12.6.5.**

In the case of a medical emergency the Investigator may contact the Medical Responsible Person at Swedish Match.

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

## 5.2 Overdose

An overdose is a dose in excess of the dose specified for each cohort in this clinical study protocol (CSP).

Over-dosing is not likely to occur in this study since all investigational products will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required (see Section 7.3.1).

Overdose should be recorded as follows:

- An overdose with associated adverse event (AE) is recorded as the AE diagnosis/symptoms on the relevant AE modules in the case report form (CRF).
- An overdose without associated symptoms is only reported in the subject's medical records.

# 6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

**Sponsor**

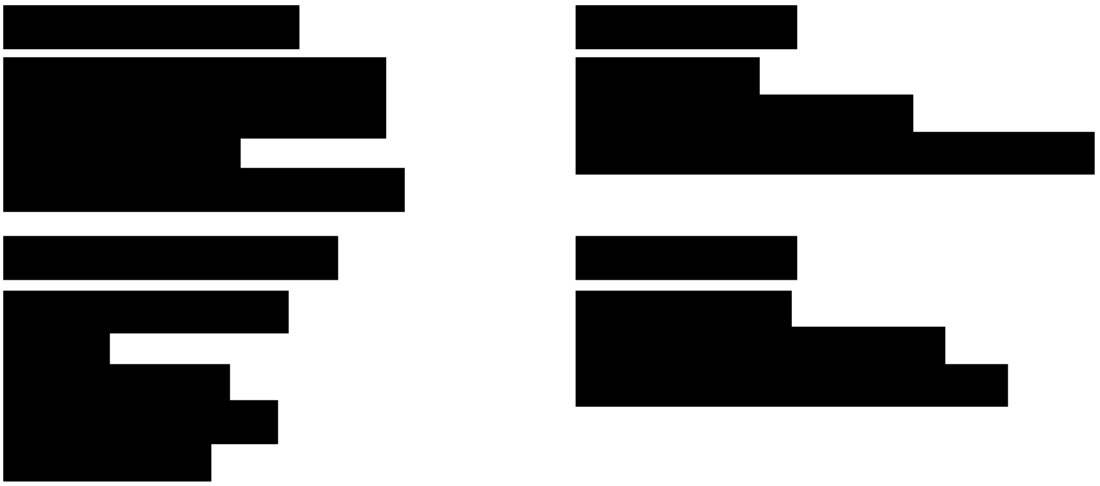
Swedish Match
SE-Box 17037
104 62 Stockholm,
Sweden
Maria Skolgata 83, 1 tr
SE-11885 Stockholm, Sweden

[redacted]

**Clinical conduct and Management**

CTC Clinical Trial Consultants AB
Dag Hammarskjölds väg 13
SE-752 37 Uppsala, Sweden

[redacted]



### 6.1.1 Investigational Product manufacturing and packaging

The investigational and reference products will be manufactured and packaged compliant with Swedish law on food production. The investigational and reference products will be transferred from the original container, weighed and individually packaged in identical sealed food approved test containers at Swedish Match analytical lab.

### 6.1.2 Identity of investigational products

Each dose of the investigational and reference products will be delivered in separate identical food approved glass containers labeled with unique identification numbers.

Signatures required are provided in Appendix 18.1.

# 7 INTRODUCTION

## 7.1 Project background

Sweden displays the lowest prevalence of smoking in Europe, particularly among males. Population surveys have indicated that snus is the most frequently used smoking cessation aid. Snus is sometimes used as a last resort for people who have failed stopping smoking with the available, pharmaceutical smoking cessation aids. Smokeless tobacco is capable of rapidly delivering nicotine to the bloodstream (Fant et al 1999), and therefore may be more satisfactory among smokers than currently available pharmaceutical nicotine products. Traditionally there has been no non-tobacco-based nicotine product on the Swedish market intended for recreational use similar to Swedish snus. Despite the vast risk differential between snus and cigarettes in terms of adverse long-term health effects including cancer, cardiovascular disease and chronic lung disease, snus remains a controversial product as it contains tobacco and is intended for recreational use. The tobacco component of snus explains why it contains measurable amounts of hazardous constituents such as potentially carcinogenic nitrosamines, albeit at very low concentrations.

Recently, a novel, non-tobacco-based nicotine product (ZYN®) has been developed. It has some features that are similar to snus: it comes in pouches with a nicotine content of 3 or 6 mg; it is used the same way as snus, that is, it is placed under the upper lip. In contrast to snus the product contains no nitrosamines or polycyclic hydrocarbons (PAHs), which are the two main classes of unwanted substances in snus that are classified as potentially carcinogenic. Other unwanted substances in ZYN® are present in comparable or lower concentrations than in snus. The toxicological safety profile of ZYN® thus represents a significant improvement over snus with the exception of the nicotine content which is only marginally lower than in snus (3 or 6 mg in ZYN® versus e g 8-12 mg in a conventional 1.0 g snus pouch).

Commercially available snus products have a nicotine content ranging between 1-2%. Previous studies (Lunell E and Curvall M 2011), have indicated that on average about 15-20% of the total nicotine content is extracted and absorbed, with large inter-individual variation. Extraction is generally not linear with pouch size: surface area, saliva penetration and diffusion factors may be important determinants of nicotine uptake.

The nicotine delivery profile of a tobacco-free product like ZYN® is probably a main determinant of its efficacy to function as an alternative to cigarettes and snus. In view of these circumstances, it is highly justified to study the nicotine delivery profile of ZYN® in comparison with commercially available snus products (which have a documented ability to replace cigarettes as a source of recreational nicotine among current tobacco consumers). The Sponsor has previously conducted studies of nicotine chewing gum with different nicotine content versus snus products. We now intend to extend those observations by comparing the ZYN® product with Swedish snus.

The main aim of the present study is to document the *in-vivo* extraction of nicotine from ZYN® pouches and the resulting uptake to the systemic blood circulation, measured as $AUC_{inf}$, based on plasma concentrations of nicotine, versus a conventional snus pouch. The extraction and plasma data will be supplemented with assessments of subjective effects of "product strength" and pulse rate measurements, both of which constitute proxies for systemic nicotine uptake.

## 7.2 Investigational products

### 7.2.1 Product characteristics

Test and reference products will be delivered in identical containers labeled with unique identification numbers.
The test product of non-tobacco-based nicotine contains 3 and 6 mg of nicotine, respectively, in a pouch.
The reference product of 1 g Swedish snus pouch contains 8 mg of nicotine.
Administration of the pouch will be between the upper lip and the gum.

## 7.3 Risk/benefit assessment

It may be considered problematic to expose research subjects to a novel nicotine delivery product the properties of which are not yet fully known. However, all research subjects are required to be daily snus users since at least one year (with an average or above snus consumption) so the participants are well acquainted with and used to the effects of nicotine. Preliminary data from the manufacturer (J. Lindholm, personal communication) indicate that the amount of nicotine extracted from the test articles is comparable to that from tobacco-based snus, despite the fact that the overall nicotine content and content of free nicotine in the ZYN® pouches, 3 and 6 mg, is lower than in conventional tobacco-based snus (8 mg). This suggests that adverse effects from the nicotine exposure from the test and reference articles are unlikely to occur among the research subjects.

Aside from the nicotine, all ingredients used in the test products are food-approved (similar to ingredients in conventional snus). The nicotine in ZYN® is of pharmaceutical grade, i.e. the same as the nicotine in nicotine replacement products (gum, lozenges, mouth spray etc.). ZYN® is currently commercially available on the U.S. and Swedish markets.

The study does not involve invasive procedures, beside the collection of venous blood samples. So far, no adverse effects have been reported associated with the use of ZYN® apart from effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia).

Pregnant women or individuals with a history of hypertension or any cardiovascular disease, who may be particularly vulnerable to nicotine exposure, are excluded from participation.

The potential adverse effects of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective counterbalanced by the potential positive effects of the novel nicotine pouch as a reduced toxicity alternative to conventional snus. 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.

### 7.3.1 Summary of risk management

Subjects will remain in the research clinic for 6 hour after the administration of the Investigational Product and will be closely monitored by medical staff.

# 8 STUDY OBJECTIVES AND ENDPOINTS

## 8.1 Primary objective(s)

To compare each subject's $AUC_{inf}$ based on plasma concentrations of nicotine after administration of one single dose of a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, to that of one single dose from a 1 g Swedish snus pouch containing 8 mg of nicotine.

### 8.1.1 Primary endpoint (s)

$AUC_{inf}$ based on plasma concentrations of nicotine after administration of one single dose of a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, to that of one single dose from a 1 g Swedish snus pouch containing 8 mg of nicotine.

## 8.2 Secondary objectives

1. To compare $AUC_{60min}$, $C_{max}$, $T_{max}$, AUCo-t and terminal half-life of a novel, non-tobacco-based nicotine pouch to that of a Swedish snus pouch.
2. To compare the estimated *in-vivo* extracted amount of nicotine from a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, respectively, with that from a 1 g Swedish snus pouch containing 8 mg of nicotine.
3. Comparison of pulse rate and subjective effects ("head buzz") after study product administration (proxy for *in vivo* nicotine uptake).
4. Adverse events

### 8.2.1 Secondary endpoints

1. $AUC_{60min}$, $C_{max}$, $T_{max}$, AUCo-t and terminal half-life of a novel, non-tobacco-based nicotine pouch to that of a Swedish snus pouch.
2. *In-vivo* extracted amount of nicotine
3. To assess the correlation between the estimates of AUC(inf) and the total amount of nicotine extracted from the ZYN® pouches.
4. Pulse rate and VAS for measure "head buzz" (head rush, "hit", feeling alert, overall "product strength"), using a 100-mm visual analogue scale (VAS) anchored with "not at all" to "extremely" at preset time points up to 60 minutes (predose, + 5min, +10 min, + 15 min, +30 min, +60 min after each dose), respectively, after study product administration (proxy for systemic uptake).
5. Collection of adverse events

# 9 INVESTIGATIONAL PLAN

## 9.1 Overall study design and schedule of events

The study will be conducted as an open, randomized, five-way cross-over, single dose administration. The study will include 18 subjects.

The subjects included will be healthy males and females aged ≥19 years who use tobacco-based snus, since ≥1 year with a weekly consumption of three or more snus cans (brands with nicotine content ≤1%) or two or more cans (brands with nicotine content >1%). Subjects who are pregnant or who have a history of hypertension or any cardiovascular disease are excluded. Subjects shall be abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.

Before entry to the study subjects undergo screening evaluations including smoking and snus use, medical history and pulse rate measurements before/after application of their usual brand of snus. The puls rate assessment will be made in abstinent condition from 8 p.m. the night before.

A pulse rate increase of ≥10 beats/min in the morning before use of any nicotine containing product will classify the subject as eligible for participation the study.

Subjects report to the clinic on separate days for the 5 experimental sessions. The subjects are instructed to abstain from snus, cigarettes or other nicotine delivery products as from 8 pm the evening before. All sessions are performed during the morning hours to facilitate abstinence. The subjects should certify abstinence before each treatment is started.

The treatments are administered as single doses in a pre-determined randomized order. The subject keeps the pouch still between the upper lip and the gum for 60 minutes and are instructed not to manipulate the pouch with the tongue or lips. The subjects are instructed not to eat, drink, chew chewing gum or brush teeth from 30 minutes before application of treatment, during application of investigational products and 30 minutes after the investigational product have been taken out. [5].

Each used pouch is collected and frozen (-20°C) pending analyses of nicotine.

**Table 1 Overall Schedule of Events**

| Assessment | Screening | Cross-over phase | Follow-up<br>Phone contact |
|---|---|---|---|
| | Visit 1[1] | Visit 2-6[2] | Visit 7 |
| | Day -14 to -1 | Day 1 followed by 1-14 day(s) of wash-out. Repeated for each dose time point | 7 Days after last dose −3/+7 days |
| Informed consent | X | | |
| Eligibility | X | X[8] | |
| Demographics | X | | |
| Prior and Concomitant medication | X | X | X |
| Medical history | X | | |
| Urine pregnancy test[3] | X | | |
| HIV, Hepatitis B and C test | X | | |
| Drug screen | X[4] | X[4] | |
| CO measurement | X | X | |
| Pulse rate | X[5] | X[6] | |
| Visual Analogue Scale | | X[6] | |
| Investigational Product administration | | X | |
| Collection of pouches | | X | |
| PK blood sampling | | X[7] | |
| Baseline Symptoms | X | X[8] | |
| Adverse Events | | X | X |

[1] Visit 1 could be performed during 2 days

[2] Refer to Schedule of Events per visit for details.

[3] Female subjects only

[4] Drug tests during the treatment period may be performed randomly.

[5] Before and 15 min after application of the subject's usual brand and amount of Swedish snus

[6] Before and 5, 10, 15, 30 and 60 min after application of the investigational products

[7] Before and 5, 10, 15, 30, 60, 90, 120, 240 and 360 min after application of the investigational products

[8] Only prior to dose administration on visit 2.

### 9.2 Rationale for study design and dose groups

Pouched Swedish snus has recently been studied in a report by Lunell and Curvall (2011). The snus investigated in that report, Swedish portion snus PSWL 1.0 g (8 mg nicotine/g), released an average 2.18 mg nicotine following use over 30 minutes. Non-tobacco-based products with similar nicotine content are investigated in the present study. The rationale for the choice of the 3 mg and 6 mg dose of the non-tobacco-based nicotine pouch is that 6 mg proved safe in a previous study (Molander L and Lunell E 2001). In view of these circumstances, it is highly justified to study the nicotine delivery profile of the non-tobacco-based nicotine pouch (ZYN®) in comparison with commercially available snus products. We thus intend to extend those observations by comparing a nicotine non-tobacco-based nicotine pouch (ZYN®) with Swedish snus, PSWL 1.0 g (8 mg nicotine/g).

## 10 STUDY POPULATION

### 10.1 Recruitment

The subjects will be recruited from a list of healthy volunteers at CTC and from advertising in media.

### 10.2 Screening and enrolment log

A screening number will be allocated to each subject undergoing screening. 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 screening 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.

All subjects who have signed the Informed Consent Form (ICF) will be assigned a screening number (S0001, S0002 and S0003 etc.). Subjects included and randomized will be assigned a subject number (101, 102 and 103 etc.).

### 10.3 Number of subjects

The study will include 18 subjects.

### 10.4 Inclusion criteria

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

1. Snus user, with a minimum weekly consumption of three or more snus cans (brands with nicotine content <1%) or two or more cans (brands with nicotine content >1%) since ≥1 year.
2. Consent to participate voluntarily and sign Informed Consent Form prior to any study procedure.
3. Healthy male/female, age ≥19.
4. Willing and able to comply with study procedures.

5. A heart rate increase ≥ 10 beats/min with first use of snus in the morning after overnight abstinence from any nicotine exposure.

### 10.5 Exclusion criteria

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

1. Smoker, defined as "smoking during the last 24 hours according to self-report and CO in exhaled air >10 ppm at clinical visits".
2. A history or presence of diagnosed hypertension or any cardiovascular disease.
3. Surgery within 6 months of the screening visit that, in the opinion of the investigator, could negatively impact on the subject's participation in the clinical study.
4. Any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the investigational product.
5. 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.
6. Pregnancy or planning to get pregnant during the study.
7. Positive screen for drugs of abuse at screening or on admission to the unit prior to administration of the investigational product.
8. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
9. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.
10. Use of any prescribed or non-prescribed medication including antacids, analgesics and herbal remedies within two weeks prior to the first administration of IP, except occasional intake of paracetamol (maximum 2 000 mg/day; and not exceeding 3 000 mg/week), at the discretion of the Investigator.
11. Plasma donation within 1 month of Screening or any blood donation/blood loss >450 mL during the 3 months prior to Screening.
12. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

### 10.6 Restrictions during the study

1. Subjects shall be abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.
2. Subjects shall abstain from smoking the last 24 before each study day.

3. The subjects are not allowed to eat or drink, or use any other mouth related procedure (e.g. tooth brushing) 30 minutes before dose administration, during application of investigational products and 30 minutes after the investigational product have been taken out. [5].
4. Other therapy considered necessary for the subject's welfare may be given at the discretion of the Investigator. All such therapy must be recorded in the Case Report Form.
5. The female volunteers are expected to be sexually abstinent or use contraceptives to prevent pregnancy during the study period
6. Consumption of grapefruit and/or grapefruit containing products is not allowed 1 week before IP dosing until last PK day.
7. Xanthine or taurine containing products/beverages, e.g. Redbull, are not allowed during the study.
8. Abstain from drugs of abuse from Screening to Follow-up visit.
9. The subjects must not donate blood or plasma during the study until three months after the Follow-up Visit.
10. Study subjects are not allowed to participate in any other clinical study during the study period.

## 10.7 Criteria for subject withdrawal

### 10.7.1 General withdrawal criteria

A subject should be withdrawn from the study treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the wish of the subject. The reason for withdrawal should be clearly described and the subject should, whenever possible, irrespective of the reason for withdrawal, as soon as possible be medically examined. Relevant samples should be obtained and all relevant assessments should be completed, preferably according to the schedule for the final assessment. The Case Report Form should be completed as far as possible and collected by the staff.

### 10.7.2 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will be asked about the reason(s) for discontinuation and the presence of any AEs. If possible he/she will be seen by the Investigator and assessed according to the procedures scheduled for the follow-up visit. Any ongoing AEs will be followed as described in Section 12.6.6.

### 10.7.1 Subject replacement

Subjects who are prematurely withdrawn from the study for any reason will not be replaced.

### 10.8 Randomization

Subjects will be assigned to the treatments using a computer-generated randomization list.

### 10.9 Blinding

The present study will be an open randomized study. Subjects will be administered each dose by the personnel according to the randomization list.

## 11 TREATMENTS

### 11.1 Identity of investigational products

**Test articles:**

**1=** ZYN Smooth containing 3 mg nicotine per portion

**2=** ZYN Smooth containing 6 mg nicotine per portion

**3=** ZYN Smooth containing 3 mg nicotine per portion (alternative manufacturing process)

**4=** ZYN Smooth containing 6 mg nicotine per portion (alternative manufacturing process)

**Reference articles:**

**5=** Swedish portion snus PSWL 1.0 g (8 mg nicotine/g)

### 11.2 Packaging and labelling

Investigational products will be delivered to the study site in identical containers labeled with unique identification numbers by Swedish Match in accordance with the randomization list.

### 11.3 Conditions for storage

The Investigational Product will be stored in the access-controlled storage area at CTC, as per storage conditions specified by the Sponsor.

### 11.4 Dispensing and accountability

Investigational Product will be dispensed as per randomization schedule by site personnel.

CTC AB and the Investigator will maintain an *Investigational Product Accountability Log* and *Investigational Product dispensing log* detailing the dates and quantities of Investigational Product received, dispensed to and used by each subject and Investigational Product returned or destroyed at the end of the study. Any discrepancies between dispensed and returned Investigational Product must be explained and documented. Products deliberately and/or accidentally destroyed by the site personnel or the subject must be accounted for.

### 11.5 Treatment administration

A single dose will be given on the morning of each study day (see specific dosing instruction).

### 11.6 Treatment compliance

All Investigational Products will be administered at the research clinic under supervision to ensure compliance.

### 11.7 Return and destruction of investigational products

Empty containers will be destroyed at the study site. The Monitor will perform final Investigational Product accountability reconciliation at the study end to verify that all unused Investigational Product is adequately returned to the Sponsor and documented.

## 12 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are detailed in the schedule of events (Table 1 Overall Schedule of Events, Section 9.1). A detailed schedule of events with assessment time points during treatment days is presented below.

### 12.1 Screening assessments

Before entry to the study subjects undergo screening evaluations including smoking and snus use, medical history and pulse rate measurements before/after application of their usual brand of snus.

### 12.2 Visits after Investigational product administration

The subjects should certify abstinence before each treatment is started. A detailed list of event after the administration of the investigational product is displayed in Table 2 below.

**Table 2 Detailed Schedule of Events, Visit 2-6.**

| EVENT | COLLECTION OF PLASMA SAMPLES | PULSE RATE | VAS ASSESSMENT | COLLECTION OF USED POUCH SAMPLE (±1 MIN) | AE INTERVIEW |
|---|---|---|---|---|---|
| *Predose* | ✓ | ✓ | ✓ | | ✓ |
| *5 min* | ✓ | ✓ | ✓ | | |
| *10 min* | ✓ | ✓ | ✓ | | |
| *15 min* | ✓ | ✓ | ✓ | | |
| *30 min* | ✓ | ✓ | ✓ | | |
| *60 min* | ✓ | ✓ | ✓ | ✓ | |
| *90 min* | ✓ | | | | |
| *2 hrs* | ✓ | | | | |
| *4 hrs* | ✓ | | | | |
| *6 hrs* | ✓ | | | | ✓ |

### 12.3 Recording of data

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

Serial plasma samples are drawn before, and at regular time intervals up to 6 hours after administration (10 samples). It is important that assessments are performed as close as possible to scheduled time. In order to achieve this, the timing priority order at a particular time point is:

1. Pulse rate
2. Collection of plasma sample
3. VAS

The time points for measurements will start from the start time of placing the snus between the upper lip and the gum. The plasma sample may be drawn with ± 5 % deviation from the time stated in the protocol.

The actual pouch sampling time should always be recorded in the CRF. Pre-dose assessments may be performed up to 10 minutes prior to dosing (if not specified in the schedule of events).

## 12.4 Demographics and other baseline characteristics

### 12.4.1 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.

### 12.4.2 Demographic information

The following demographic data will be recorded: gender, age and ethnic origin.

### 12.4.3 Medical/surgical history

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

### 12.4.4 HIV and Hepatitis B/C

Subjects will be tested for HIV and hepatitis B/C prior to inclusion into the study in order to protect personnel handling the blood samples.

### 12.4.5 Urine drug screen

Urine will be screened for drugs of abuse at screening using the Alere™ Drug Screen Test Panel. Additional random tests can be performed during the study period.
The following substances will be included in the screen panel:
Amphetamine, Barbiturates, Benzodiazepines, Buprenorphine, Clonazepam, Cocaine, Fentanyl, Ketamine, Marijuana (Tetrahydrocannabinol [THC]), Methadone, Methamphetamine, Methylenedioxymethamphetamine (MDMA), Morphine, Opiate, Oxycodone, Phencyclidine, Propoxyphene, Tramadol, Tricyclic antidepressants (TCA)

### 12.4.6 Pregnancy urine test

Pregnancy urine test will be performed at screening visit (females only).

### 12.4.7 CO test

Measurement of Carbonmonoxide in exhaled air will be performed at visits to the clinic.

### 12.4.8 Prior and concomitant medication

Prior medication, medication 2 weeks prior to screening, will be obtained by interview and documented in the subjects CRF.

Medications are classified as prior if the stop date was before or on the day of the first dose administration and as concomitant if ongoing at, and stopped after the first dose administration or started after the first dose administration.

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

### 12.4.9 Baseline symptoms

A *baseline symptom* is an event in a clinical study subject that occurs after he/she signed the informed consent form (ICF) up until the first administration of Investigational Product (*i.e.* during the screening period

## 12.5 Study assessments

### 12.5.1 Pharmacokinetics assessments

Nicotine plasma concentrations are determined at preset time points, before *(0), 5, 10, 15, 30 and 60* minutes, and 1.5, 2, 4 and 6 hours after administration. Frozen plasma samples collected for nicotine determinations will be shipped to a certified contract laboratory The analysis of the plasma samples will be performed by a validated LC-MS/MS assay at ABS Laboratories Ltd, UK. To quantify nicotine a multilevel calibration at eight concentrations will be performed over a range of 0.5 to 100 ng/mL. The calibration line will be fitted by means of linear regression weighted by 1/concentration2. The samples will be assayed once. Incurred sample reproducibility will be performed according to the EMA and FDA guidelines so that 10% of the analysed study samples up to 1000 will be reanalysed and then 5% of the number above 1000. The analysis batch acceptance criteria will be: The calibration standards must have a back-calculated accuracy within 100 ±15%, except at the lower limit of quantification (LLOQ) where it must be within 100 ±20%. The standard curve must be constructed from at least three quarters (i.e. 12) of the calibration standards, excluding the zero concentration calibration standards. Duplicate quality control samples at low, medium and high concentrations will be included in each analysis batch. The accuracy of at least two thirds of the quality control samples must be within 100 ±15%. Half of the quality control samples at each concentration must be within 100 ±15%. At least half of the blank samples with internal standard and half of the blank samples without internal standard, placed immediately before the calibration standards, must be free of interference. Overall two thirds of the total number of blank samples must be free of interference. Interference is defined as a detectable response, at the retention time of the analyte, greater than 20% of the mean response of the lowest concentration (LLOQ) standards. WinNonlin computer program (Certara Corp., USA) will be used for pharmacokinetic calculations. Main variables will be $AUC_{inf}$ , $C_{max}$, $T_{max}$.

### 12.5.2 Visual Analogue Scale and Vital signs

VAS for measure ”head buzz” (head rush, ”hit”, feeling alert, overall ”product strength”), using a 100-mm visual analogue scale (VAS) anchored with ”not at all” to ”extremely” at preset time points up to 60 minutes, after study product administration (as a proxy for systemic uptake). VAS assessment will be performed with a VAS-ruler. Pulse rate will be measured with automatic device in sitting position after 10 minutes of rest.

### 12.5.3 Collection of pouches and analysis

Pouches for the determination of nicotine after administration of the Investigational Product will be collected after 60 minutes (see Overall study design and schedule of events in Section 9.1). The following time window will apply for the pouch sampling:

- ± 1 min.
- The date and time of collection of each pouch will be recorded in the CRF.

All the collected pouches will be collected and frozen immediately at -20°C.

Pouches for extraction of nicotine will be analyzed by Swedish Match. Pouches from all evaluable subjects excluding withdrawn or dropout subjects will be analyzed.

## 12.6 Adverse events

The Principal Investigator is responsible for ensuring that all medical staff involved in the study is familiar with the content of this section and the content of the CTC standard operating procedures (SOPs).

### 12.6.1 Event definitions

#### 12.6.1.1 Adverse event

An Adverse Event (AE) is any untoward medical occurrence in a subject or trial subject to whom a drug is administered or in whom a medical device is used: The event does not necessarily have a causal relationship with that treatment or usage.
Adverse Events include the following:

a) All suspected adverse reactions to the study products (such as excess salivation, nausea, vomiting, hiccups, head ache, palpitations, dyspepsia).
b) Apparently unrelated illnesses, including the worsening of a pre-existing illness (see 'Pre-existing Conditions' below).
c) Injury or accidents.
d) Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with a clinical event already reported. Laboratory abnormalities associated with a clinical event (e.g. elevated liver enzymes in a subject with jaundice) should be described under 'Comments' on the report of the clinical event rather than be listed as a separate adverse event.

#### 12.6.1.2 Baseline symptom

In this trial, a baseline symptom (i.e. a disorder present before the AE reporting period started and will be noted on the pre-treatment medical history/physical examination form) should not be reported as an AE unless the condition worsens or episodes increase in frequency during the AE reporting period.

#### 12.6.1.3 Procedures

Diagnostic and therapeutic invasive and non-invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE and the resulting appendectomy be noted under 'Comments'.

#### 12.6.1.4 Serious adverse event

An AE that meets one or more of the following criteria is classified as serious:

- Death
- Life-threatening (i.e. immediate risk of death)
- In-subject hospitalization or prolongation of existing hospitalization
- Permanent or significant impairment of function or permanent damage to a body structure or intervention is required to prevent permanent impairment or damage
- Cancer
- Any other AE that the investigator or company judges to be serious, or which is defined as serious by the regulatory agency in the country in which the adverse event occurred.

### 12.6.2 Adverse Event assessment definitions

#### 12.6.2.1 Assessment of severity/intensity

The grading of the severity/intensity of AEs will follow the CTCAE v4.03. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the CRF:

| | |
|---|---|
| *Grade 1* | Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| *Grade 2* | Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*. |
| *Grade 3* | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self- care ADL**. |
| *Grade 4* | Life-threatening consequences; urgent intervention indicated. |
| *Grade 5* | Death related to AE. |

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*

***Self- care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

#### 12.6.2.2 Assessment of causal relationship

The Investigator must assess the *causal relationship* between an AE and the Investigational Product using the definitions below and record it on the *Adverse Event Form* in the CRF as well as on the *Serious Adverse Event Report Form*, if applicable:

- *Probable* – the AE has a strong temporal relationship to the Investigational Product or recurs on re-challenge, and another etiology is unlikely or

significantly less likely

- *Possible* – the AE has a suggestive temporal relationship to the Investigational Product, and an alternative etiology is equally or less likely
- *Not related* – the AE has no temporal relationship to the IMP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the Investigational Product and the AE).

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

For a baseline symptom, a causality assessment is not relevant.

#### 12.6.2.3 Assessment of outcome

The Investigator must assess the *outcome* of an AE using the definitions below and record it on the *Adverse Event Form* in the CRF:

- *Recovered* – the subject has recovered completely, and no symptoms remain.
- *Recovering* – the subject's condition is improving, but symptoms still remain.
- *Recovered 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* – the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
- *Death*

### 12.6.3 Collecting adverse events

AEs (including baseline events) 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

Collection of baseline events starts after the subject signs the ICF and continues until the first administration of Investigational Product.

AE collection starts with administration of the Investigational Product (*i.e.* only TEAEs will be collected and recorded in the CRF) and continues until the last follow-up assessment. Any AE with start date on the day of first Investigational Product administration must be recorded with start time.

At the Follow-up Visit, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

### 12.6.4 Recording adverse events

AEs (including baseline events) must be recorded on an *Adverse Event Form* in the CRF. 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 IMP; action taken, and outcome.

If the AE is serious, this must be indicated in the CRF. Furthermore, the Investigator must fill out the *Serious Adverse Event Report Form* and report the SAE to the Sponsor as described in Section 12.6.5.

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.

If the severity/intensity of an AE increases a new *Adverse Event Form* must be completed in the CRF.

### 12.6.5 Reporting serious adverse events

All AEs should be followed until they are resolved, or the subject's participation in the trial ends. Instructions for reporting changes in an ongoing AE during a subject's participation in the trial are provided in the instructions that accompany the CRF AE forms.
In addition, all serious AEs and those non-serious events assessed by the investigator as possibly related to the investigational medication/product should continue to be followed even after the subject's participation in the trial is over. Such events should be followed until they are resolved or until the Investigator assesses them as "chronic" or "stable". Resolution of such events is to be documented on the appropriate CRF.

The Investigator must report SAEs to the Sponsor immediately (within 24 hours) after becoming aware of them, by contacting:

[redacted]

The same information must also be sent to the CTC SAE email inbox: [redacted]

To report SAEs, the *Serious Adverse Event Report Form* for clinical studies provided must be used. The first report should contain as much information as possible. The initial report is to be followed by submission of more detailed and additional AE information within 5 working days of the event using the same form. If unexpected, SAEs are also to be reported immediately to the responsible Independent Ethics Committee.

The Sponsor or a delegate will assume responsibility for reporting SAEs in accordance with local regulations.

The Sponsor is responsible for informing the Investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

#### 12.6.6 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 the follow-up assessment, whichever comes first. At the Follow-up Visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded. AEs assessed as stable by the Investigator at the last Follow-up 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, stabilized, 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.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment must be handled in the same manner as SAEs occurring during the study. These SAEs will be reported to the Sponsor.

#### 12.6.7 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the study treatment 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 any of the Investigational Products 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.

### 12.7 Appropriateness of measurements

Measurements of nicotine extraction, pulse rate and Visual Analogue Scales are standard assessments in nicotine research.

Standardized methods for measurements of safety and tolerability will be used.

## 13 PROCEDURES FOR SAMPLING AND ANALYSIS OF NICOTINE

### 13.1 PK plasma samples

#### 13.1.1 Sample collection

Blood samples (5 mL) for the determination of plasma concentrations of nicotine will be collected at the specified time-points on Visits 2 to 6. The plasma fraction of the samples will be collected and divided into two cryo tubes containing approx.1 ml each. For further details refer to the separate lab manual. The date and time of collection of each sample will be recorded in the eCRF.

The samples will be registered in a tissue-bank 893 at CTC and stored at -70°C until analyzed. The samples will be disposed after the Clinical Study Report (CSR) has been finalized.

#### 13.1.2 Analysis of PK plasma samples

Samples for determination of plasma concentrations of nicotine will be analysed by ABS Laboratories LTD (United Kingdom), by means of a validated LC-MS/MS method. The details of the analytical method used will be described in a separate bioanalytical report.

Samples will be collected, stored, and shipped to the laboratory for analysis in accordance with study-specific instructions.

For additional information on the analysis of PK parameters, refer to Section 12.5.1.

### 13.2 Nicotine extraction

#### 13.2.1 Sample collection

The nicotine content per portion of used and unused pouches, respectively, will be estimated. Each used pouch is collected and frozen (-20°C) pending analysis of nicotine. Frozen samples of used pouches collected for nicotine determinations will be shipped to Swedish Match laboratories.

#### 13.2.2 Analysis of nicotine in pouches

Nicotine in used and unused pouches will be analyzed at the Swedish Match laboratories using a validated method.

## 14 ETHICAL AND REGULATORY REQUIREMENTS

### 14.1 Ethical conduct of the study

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, and later revisions.

A link to the Declaration of Helsinki is included in 18.2.

### 14.2 Ethics and regulatory review

It may be considered problematic to expose research subjects to a novel nicotine delivery product the properties of which are not yet fully known. However, all research subjects are required to be daily snus users since at least one year (with an average or above snus consumption) so the participants are well acquainted with and used to the effects of nicotine. Preliminary data from the manufacturer (J. Lindholm, personal communication) indicate that the nicotine extraction from the test articles is comparable to that from tobacco-based snus, despite the fact that the overall nicotine content and content of free nicotine in the ZYN® pouches, 3 and 6 mg, is lower than in conventional tobacco-based snus (8 mg). This suggests that adverse effects from the nicotine exposure from the test and reference articles are unlikely to occur among the research subjects.

Aside from the nicotine, all ingredients used in the test products are food-approved (similar to ingredients in conventional snus). ZYN® is currently commercially available on the U.S. and Swedish markets. The nicotine in ZYN® is of pharmaceutical grade, i.e. the same as the nicotine in nicotine replacement products (gum, lozenges, mouth spray etc.).

So far, no adverse effects have been reported associated with the use of ZYN® apart from well-known effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia).

Pregnant women or individuals with a history of hypertension or any cardiovascular disease, who may be particularly vulnerable to nicotine exposure, are excluded from participation.

The study does not involve invasive procedures, besides collection of venous blood samples from an antecubital vein using an inserted cannula (Venflon®).

The theoretical adverse effects of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective counterbalanced by the potential positive effects of the novel nicotine pouch as a low-toxic alternative in comparison with cigarettes or conventional snus among current tobacco users.

The study will not be started until approval of the protocol, the Subject Information and the Informed Consent Forms have been obtained from the Independent Ethics Committee in Uppsala, Sweden. It is the responsibility of the Investigator to forward a copy of the written approval and, where possible, a list of the members, their titles or occupations, and their institutional affiliations, to CTC/the Sponsor. The approval should include a study identification and the date of review. The study will not be started until receipt by CTC of written approval from the IEC.

### 14.3 Subject information and consent

It is the responsibility of the Investigator or designee to give each subject prior to inclusion in the study, full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. The subjects must be informed about their right to withdraw from the study at any time. Written subject information should be given to each subject before enrolment. The written subject information must not be changed without prior discussion with CTC/the Sponsor. All subjects are required to provide informed consent prior to any study procedures. Furthermore, it is the responsibility of the Investigator or designee to obtain signed Informed Consent Form from all subjects prior to inclusion in the study.

The signed Informed Consent Form should be filed by the Investigator or designee for review by the Monitor. The Investigator will confirm receipt of the Informed Consent Form from each subject by signing the appropriate page of the Case Report Form.

### 14.4 Subject data protection

The Investigator should keep a subject identification list not to be available to the Sponsor, including sufficient information to link records, i.e. CRFs and hospital records. The subjects should be informed that the data will be stored and analyzed by computer, that Swedish and local regulations for the handling of computerized data will be followed and described in the written subject information and that identification of individual subject data will only be

possible for the Investigator. Furthermore, the subjects should be informed about the possibility of inspection of relevant parts of the records by representatives of CTC AB and/or Authorities.

### 14.5 Changes to the approved clinical study protocol

Any variation in procedure from that specified in the Final Study Protocol may lead to results of the trial being questioned and in some cases rejected. Any proposed protocol change must therefore be discussed with and approved by the Sponsor and submitted for Independent Ethics Committee approval or notification. Any protocol change should be documented in a Protocol Amendment.

### 14.6 Audits and inspections

Authorized 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, analyzed, and accurately reported according to the protocol, ICH-GCP guidelines and any applicable regulatory requirements.

### 14.7 Insurance

The Sponsor is liable under law and in accordance with generally accepted standards for unexpected injuries, including death, that the use of the study drug may cause subjects.

The Sponsor will indemnify and hold the Investigator as well as any hospital, institution, ethics committee or the alike, harmless from any claims for damages caused by such injuries but only to the extent that the claim is not caused by gross negligence or failure to comply with the protocol and/or governmental regulation by the indemnified.

The Sponsor will require the Investigator to indemnify and hold the Sponsor harmless from any claim caused by gross negligence and/or failure to comply with the protocol and/or governmental regulation by the Investigator.

The Investigator agrees to notify the Sponsor whenever he becomes aware of a claim or action and to co-operate with and authorize the Sponsor to carry out sole management of such claim or action.

The Sponsor’s responsibility is covered by product liability insurance. The insurance also covers the Sponsor’s liability under law and generally accepted liability standards within industry toward any third parties, including subjects, as Sponsor of the Study. The Investigator’s responsibility is covered by liability insurance for scientific studies in human subject. 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 clinic. 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) utilized.

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, including detailed knowledge of and training in all procedures to be followed.

Curriculum vitae will be collected from all personnel and be kept in the Trial Master File.

## 15.2 Clinical monitoring

The study site will be monitored periodically during the study, as agreed with the Investigator. The Monitor will ensure that all aspects of the protocol are followed, including the randomization procedure, the accurate recording of results, the reporting of Adverse Events, Product Accountability and record keeping.

Furthermore, it will be verified that the clinical facilities remain accurate, and that the Case Report Forms are in agreement with source data. For this purpose, the Monitor will be given access to hospital records, original laboratory data, etc., as far as they relate to the study, without jeopardizing subject integrity and as agreed with the Investigator prior to the study. Case Report Forms for all included subjects will be made available to the Monitor for review and collection as agreed with the Investigator. It is important that the Investigator and other relevant personnel are available during the monitoring visits and possible audits and that sufficient time is devoted to the process

## 15.3 Source data document

Monitoring visits will be made during the trial, to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data on CRFs.

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

The Investigator should guarantee access to source documents to the Monitor 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 time table and end of study

Ethics committee application will be submitted in October 2017. Performance of clinical part of study is expected to be finished in the beginning of 2018.

The end of the clinical part of the study is defined as the last visit of the last subject participating in the study.

### 15.6 Discontinuation of the study

The Sponsor reserves the right to discontinue the study prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must call in all participating subjects. At this visit, all delivered unused study products and other study materials must be collected and all CRFs be completed as far as possible.

### 15.7 Reporting and publication

#### 15.7.1 Clinical study report

A summarizing report should be submitted to the applicable IEC within 12 months after completion of the study.

A clinical study report (CSR), in compliance with ICH E3; *Structure and content of clinical study reports*, describing the conduct of the study, *the statistical analysis 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

All information not previously published concerning the test product and the Sponsor's research, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and should remain the sole property of the Sponsor. The Investigator agrees to use this information only in connection with this study and will not use it for other purposes without the written permission from the Sponsor.

#### 15.7.3 Publication

It is agreed that the Sponsor has the ownership of all results. Before publication, if publication is agreed upon, the Sponsor will be given the opportunity to review and comment upon the manuscript. The time for review should not exceed 30 days after receipt of the manuscript. If the Investigator has not submitted the results for publication within 6 months after completion of the final CSR, the Sponsor will have the right to publish. In this case the Investigator will be given 30 days to review and comment on the manuscript prior to submission to the publisher.

### 15.8 Archiving

To enable any further evaluations and/or audits from Health Authorities/the Sponsor, the Investigator agrees to keep records, including the identity of all participating subjects, all original signed Informed Consent Forms, copies of all CRFs and detailed records of drug

disposition. To comply with international regulations, the records should be retained by the Investigator for 15 years.

# 16 DATA MANAGEMENT

## 16.1 Case report form

Data will be collected in CRFs specifically designed for this study. The Investigator or an authorized person will record subject data in the CRF in a precise and accurate manner. Abbreviations should not be used. The Investigator is responsible for the data entered and will sign off the CRF at the end of the study. The data should be recorded as soon as they are generated. CRF entries must be made with an archive resistant pen. Any correction should be marked with a single bar through the error and the correct information should be written next to it. All corrections must be initialed and dated. Correction fluid must not be used. Only persons authorized by the Investigator are allowed to make entries to the CRF.

## 16.2 Database management plan and database design

Detailed information on data management will be described in a study-specific DMP.

The person entering data into the database is not allowed to attempt any personal interpretation or to make any decisions on the data other than self-evident corrections as listed in the study-specific Data Entry Instructions or Data Handling Report. Single data entry type will be applied.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of manual reviewing during data entry and computerized edit checks and queries for identifying data values that are outside the allowed range, protocol violations, incomplete or inconsistent. The Data Validation Plan (DVP) specifies the checks that are to be performed on subject data for the study. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

## 16.3 External data

External data consists of data that is not recorded in CRFs. Data may be received in electronic format or 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. Any electronically transferred data must contain origin, date created, date sent and number of records at minimum.

## 16.4 Medical encoding

Medical encoding will be done by trained personnel at CTC. AEs and medical history verbatim terms are encoded using the Medical Dictionary of Regulatory Activities (MedDRA), latest version available when approving the DMP.

Prior and concomitant medications will be coded according to the WHO Anatomic Therapeutic Chemical (ATC) classification system.

All coding will be approved by CTC.

### 16.5 Database lock

When all data have been entered, discrepancies solved the database will be locked and the data will be analyzed.

## 17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The following is an outline of the statistical methodology that will be used to analyze this study. A more detailed description will be provided in a separate statistical analysis plan (SAP) that may also include additional exploratory analyses not explicitly mentioned in the following sections. The SAP will be finalized before closure of the study database and deviations from the SAP will be reported and justified in the clinical study report.

### 17.1 General

Continuous data will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), minimum and maximum value.

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.

A significance level of 5% with two-sided tests will be used in all comparisons.

The test articles below will in all analyses be compared to the reference product.

Any comparisons between the test articles will be described in the SAP.

**Test articles:**

**1=** ZYN Smooth containing 3 mg nicotine per portion

**2=** ZYN Smooth containing 6 mg nicotine per portion

**3=** ZYN Smooth containing 3 mg nicotine per portion (alternative manufacturing process)

**4=** ZYN Smooth containing 6 mg nicotine per portion (alternative manufacturing process)

**Reference articles:**

**5=** Swedish portion snus PSWL 1.0 g (8 mg nicotine/g)

### 17.2 Determination of sample size

The primary endpoint is nicotine extraction. The study will include 18 subjects. A previous study [Lunell E & Curvall M 2011] has made the calculation of sample size possible. Nicotine extraction from a 1 g Swedish portion snus (PSWL) containing 8mg nicotine/pouch was estimated at 2.18±0.92 mg per portion. Under the assumption of a complete dissolution and *in-vivo* extraction of the 3 and 6 mg ZYN® products, respectively, versus the 2.18±0.92 mg nicotine, and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and alpha=0.05. The randomization will be performed using Latin Squares approach.

## 17.3 Analysis data sets

### 17.3.1 Full analysis set

The FAS will consist of all subjects who have been randomized and received at least one dose of IMP.

### 17.3.2 Per protocol analysis

The PP population will consist of all subjects who have been randomized, completed the study period and without any major protocol violations. All violations will be presented and discussed at the clean file meeting.

The baseline and safety data will be presented using the FAS population. All data regarding extraction of nicotine will use the PP population.

## 17.4 Description of study population

### 17.4.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight and height will be presented by dose group.

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

Medical/surgical history and prior/concomitant medications will be presented by descriptive statistics by dose group.

### 17.4.3 Treatment compliance

The number of subjects treated in each by dose group will be tabulated.

## 17.5 Analysis of primary endpoint

$AUC_{inf}$ based on plasma concentrations of nicotine after administration of one single dose of a novel, non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, to that of one single dose from a 1 g Swedish snus pouch containing 8 mg of nicotine will be described using summary statistics and non-parametric signed Wilcoxon rank sum test for within subject difference.

## 17.6 Analysis of secondary endpoints

The mean + SD extracted dose of nicotine from each pouch, will be calculated. The extracted dose of nicotine will be analyzed using the signed Wilcoxon rank sum test and Students t-test for within subject difference. The correlation between the AUC and the total amount of nicotine extracted from the pouch will be analyzed using Proc corr. in SAS.

The mean + SD of AUCinf based on plasma concentrations of nicotine after administration of of each pouch, will be calculated. AUC60min, Cmax, Tmax, AUCo-t and terminal half-life will also be calculated.

AUC60min, Cmax, Tmax, AUCo-t and terminal half-life of a novel, non-tobacco-based nicotine pouch to that of a Swedish snus pouch will be described using summary statistics and analyzed using signed Wilcoxon rank sum test for within subject difference.

Pulse rate and VAS for measure of subjective ”head buzz” (head rush, ”hit”, feeling alert, overall ”product strength”), will be summarized by treatment and period using descriptive statistics.

The intra subject difference of pulse rate will be analyzed using the signed Wilcoxon rank sum test and Students t-test for within subject difference.

The intra subject difference of VAS scales for “head buzz” (head rush, ”hit”, feeling alert, overall “product strength”) will be analyzed using the signed Wilcoxon rank sum test and Students t-test for within subject difference.

All AE data will be fully listed by Investigator terms and MedDRA Preferred Term (PT). AE data will be summarized by System Organ Class (SOC) and PT.

## 17.7 Statistical/analytical issues

### 17.7.1 Adjustments for covariates

No adjustments for covariates will be performed.

### 17.7.2 Handling of dropouts or missing data

Missing data will not be imputed.

### 17.7.3 Multiple comparison/multiplicity

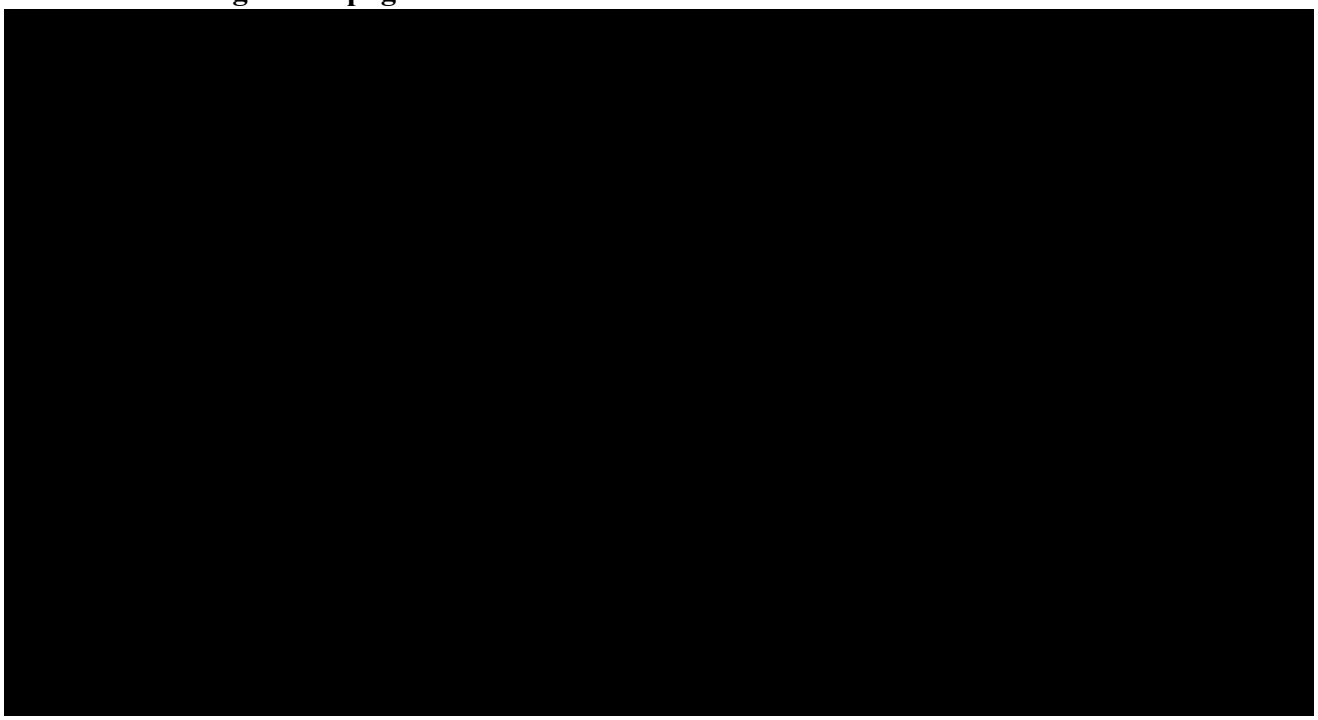
Even though many pairwise comparison will be made, will no p-value adjustments will be enforced. However, a medical/clinical judgement will be applied to all significance tests, in order to avoid any conclusions based solely on statistical significance and without any clinical relevance.

### 17.7.4 Examination of subgroups

No subgroup analysis will be performed.

# 18 APPENDICES

## 18.1 Signature page



### 18.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf

## 19 REFERENCES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3. Lunell E and Curvall M. Nicotine Delivery and Subjective Effects of Swedish Portion Snus Compared With 4 mg Nicotine Polacrilex Chewing Gum. *Nicotine Tob Res* 2011;13 (7): 573-578.
4. Chow S-C, Shao J and H Wang (2003) Sample Size Calculations in Clinical Research. Marcel Dekker, New York, pp 102-103.
5. Henningfield JE, Radzius A, Cooper TM, Clayton RR. Drinking coffee and carbonated beverages blocks absorption of nicotine from nicotine polacrilex gum. *JAMA 1990;264:1560-4.*