
Clinical Study Protocol

Investigational Product	ZYN®
Study Code	SM 17-01
Protocol Version and Date	Final 26 June 2017

STUDY TITLE

The *in-vivo* extraction of nicotine and flavor compounds from 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, fourteen-way cross-over, single dose administration. The study will include 20 subjects.
Test products and dosage	<p>1= ZYN® Smooth containing 3 mg nicotine per portion - 15 min administration.</p> <p>2= ZYN® Smooth containing 3 mg nicotine per portion - 60 min administration.</p> <p>3= ZYN® Smooth containing 6 mg nicotine per portion - 15 min administration.</p> <p>4= ZYN® Smooth containing 6 mg nicotine per portion - 60 min administration.</p> <p>5= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 15 min administration.</p> <p>6= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 60 min administration.</p> <p>7= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 15 min administration.</p> <p>8= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 60 min administration.</p> <p>9= ZYN® Wintergreen containing 3 mg nicotine per portion (Nicotine analysis) - 60 min administration.</p> <p>10= ZYN® Wintergreen containing 3 mg nicotine per portion (Flavor analysis) - 60 min administration</p> <p>11= ZYN® Peppermint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min</p>

administration.

12= ZYN® Spearmint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min administration.

Comparator product and dosage

13= Swedish portion snus PSWL 1.0 g(8 mg nicotine/g) - 15 min administration.

14= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) - 60 min administration.

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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	
The <i>in-vivo</i> extraction of nicotine and flavor compounds from 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	
SM 17-01	
Study period	
Estimated date of first subject enrolled: Q3 2017	
Estimated date of last subject completed: Q4 2017	
Principal Investigator	
Jan Erik Berglund, MD, PhD CTC Clinical Trial Consultants AB	
Study design	
Open, randomized, fourteen-way cross-over, single dose administration.	
Objectives	
<u>Primary objective(s)</u>	
To compare the estimated <i>in-vivo</i> extracted dose 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.	
<u>Secondary objectives</u>	
Comparison of pulse rate as a surrogate endpoint, for systemic uptake of nicotine.	
Comparison of each subject's rating of subjective effect ("head buzz") and pulse rate.	
To estimate the <i>in-vivo</i> extracted dose of flavor components to assess the overall flavor exposure from the non-tobacco based nicotine pouch.	
Adverse event	
Number of subjects planned	
The study will include 20 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 $\leq 1\%$) 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 14 experimental sessions. The subjects are instructed to abstain from snus, cigarettes or other nicotine delivery products as from 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 15 or 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 dose administration [5].

Each used pouch is collected and frozen (-20°C) pending analyses of nicotine and flavor components. 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 - 15 min administration.

2= ZYN® Smooth containing 3 mg nicotine per portion - 60 min administration.

3= ZYN® Smooth containing 6 mg nicotine per portion - 15 min administration.

4= ZYN® Smooth containing 6 mg nicotine per portion - 60 min administration.

5= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 15 min administration.

6= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 60 min administration.

7= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 15 min administration.

8= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 60 min administration.

9= ZYN® Wintergreen containing 3 mg nicotine per portion (Nicotine analysis) - 60 min administration.

10= ZYN® Wintergreen containing 3 mg nicotine per portion (Flavor analysis) - 60 min administration

11= ZYN® Peppermint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min administration.

12= ZYN® Spearmint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min administration.

<p>Reference articles:</p> <p>13= Swedish portion snus PSWL 1.0 g(8 mg nicotine/g) - 15 min administration.</p> <p>14= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) - 60 min administration.</p>
<p>Duration of treatment</p> <p>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 15 or 60 minutes.</p>
<p>Duration of subject's involvement in the study</p> <p>Each Subject will participate in the study approximately 55-98 days.</p>
<p>Efficacy assessments</p> <p>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.</p> <p>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.</p>
<p>Safety assessments</p> <p>Adverse Events will be collected starting with administration of the Investigational Product until the last follow-up from the first dose.</p>
<p>Statistical methods</p> <p>The study will include 20 subjects. A previous study (Lunell E & Curvall M 2011) has informed 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 $\alpha=0.05$.</p>

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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
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

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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. One explanation for the record-low low smoking prevalence is that snus has replaced cigarettes as the tobacco product of choice among many male and some female smokers. Population surveys have also 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 unwanted 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).

When comparing the nicotine content of different nicotine-containing products such as Swedish snus and Nicotine Replacement Therapy (NRT), it is important to consider that the nicotine extraction and uptake varies considerably depending on product type and formulation.

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: it is larger with small compared to larger pouches, which suggests that surface area, saliva penetration and diffusion factors may be more important determinants of nicotine uptake than pouch weight. The nicotine delivery profile of a product is probably a main determinant of its efficacy to function as an alternative to cigarettes among current smokers. 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 current study is focused on the in vivo extraction of nicotine from ZYN® pouches versus a conventional snus pouch. Previous data indicate that the extracted amount of nicotine from a

snus pouch can be used as a proxy for systemic nicotine uptake (Erik Lunell, personal communication). This observation probably helps to explain the mentioned large inter-individual variation in nicotine uptake among snus users (which may be due to constitutional differences in saliva production). In contrast to a formal nicotine uptake study an in vivo extraction study does not involve invasive procedures. The extraction data will be supplemented with assessments of subjective effects of “product strength” and pulse rate measurements, both of which also 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.

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 study does not involve invasive procedures.

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 in comparison with cigarettes or conventional snus among current tobacco users. 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 1 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 the estimated *in-vivo* extracted dose 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.

8.1.1 Primary endpoint (s)

The extracted dose of nicotine from each portion, will be calculated by subtracting the residual amount after use from the mean of 10 unused portions

8.2 Secondary objectives

1. Comparison of pulse rate as a surrogate endpoint, for systemic uptake of nicotine.
2. Comparison of each subject's rating of subjective effect ("head buzz") and pulse rate.
3. To estimate the *in-vivo* extracted dose of flavor components to assess the overall flavor exposure from the non-tobacco based nicotine pouch.
4. Adverse event

8.2.1 Secondary endpoints

1. Pulse rate predose, + 5min, +10 min, + 15 min, +30 min, +60 min after each dose
2. 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 30 and 60 minutes, respectively, after study product administration (as a proxy for systemic uptake).
3. The extracted dose of flavor components
4. Collection of adverse event

9 INVESTIGATIONAL PLAN

9.1 Overall study design and schedule of events

The study will be conducted as an open, randomized, fourteen-way cross-over, single dose administration. The study will include 20 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 $\leq 1\%$) 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.

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 14 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 15 or 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 dose administration [5].

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

Table 1 Overall Schedule of Events

Assessment	Screening	Cross-over phase	Follow-up Phone contact
	Visit 1 ¹	Visit 2-15 ²	Visit 16
	Day -14 to -1	Day 1 followed by 1-5 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 (re-check)	
Demographics	X		
Prior and Concomitant medication	X	X	X
Medical history	X		
Urine pregnancy test ³	X		
Drug screen	X	X ⁴	
Pulse rate	X ⁵	X ⁶	
Visual Analogue Scale		X ⁶	
Investigational Product administration		X	
Collection of pouches		X	
Baseline Symptoms	X		
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 will 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

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 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.

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 20 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 $\leq 1\%$) 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 . Female subjects should have a negative pregnancy test.
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. A history or presence of diagnosed hypertension or any cardiovascular disease.
2. 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.

3. Any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the drug.
4. Subjects who are pregnant.

10.6 Restrictions during the study

Subjects shall be abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.

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 and for the duration of the study procedures. [5].

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.

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, they 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 except the occurrence of TEAEs assessed as possibly or probably related to study treatment may be replaced during the course of the study.

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 - 15 min administration.

2= ZYN® Smooth containing 3 mg nicotine per portion - 60 min administration.

3= ZYN® Smooth containing 6 mg nicotine per portion - 15 min administration.

4= ZYN® Smooth containing 6 mg nicotine per portion - 60 min administration.

5= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 15 min administration.

6= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 60 min administration.

7= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 15 min administration.

8= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 60 min administration.

9= ZYN® Wintergreen containing 3 mg nicotine per portion (Nicotine analysis) - 60 min administration.

10= ZYN® Wintergreen containing 3 mg nicotine per portion (Flavor analysis) - 60 min administration

11= ZYN® Peppermint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min administration.

12= ZYN® Spearmint containing 3 mg nicotine per portion (Nicotine and Flavor analysis) - 60 min administration.

Reference articles:

13= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) (15 min administration).

14= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) (60 min administration).

11.2 Packaging and labelling

Investigational products will be delivered to the study site in identical containers labelled 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 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 approximately 8.00 a.m. on 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

Any unused Investigational Product will be frozen immediately at -20 °C and be returned to the Sponsor according to the instructions in section 13.1. 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 ([Table 2 Detailed Schedule of Events, visit 2-15](#)) 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. The pulse rate measurements at visit 1 are performed to ensure that the subject gets a pulse rate increase of ≥ 10 beats/min following the first dose of nicotine in the morning. Some people get only a minor pulse rate increase, depending on individual factors such as age.

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 2-15.

EVENT	PULSE RATE	VAS FORM	COLLECTION OF USED POUCH SAMPLE (±1,5 MIN)	AE INTERVIEW
<i>Predose</i>	✓	✓		✓
<i>5 min</i>	✓	✓		✓
<i>10 min</i>	✓	✓		✓
<i>15 min</i>	✓	✓	✓ ¹	✓
<i>30 min</i>	✓	✓		✓
<i>60 min</i>	✓	✓	✓ ¹	✓

¹ Removal of the Investigational product according to the randomization list

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.

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. VAS

The time points for measurement of pulse rate will start from the start time of placing the snus between the upper lip and the gum.

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

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

12.4.4 Urine drug screen

Urine will be screened for drugs of abuse at screening using the AlereTM 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)

12.4.5 Pregnancy urine test

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

12.4.6 Prior and concomitant medication

Prior medication (eg 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, total daily dose, unit, start and stop dates, reason for use if consistent with the definition of an AE) must be recorded. All changes in medication should be noted in the CRF.

12.4.7 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). These events are not regarded as AEs and should not be recorded in the AE log in the CRF.

12.5 Study assessments

12.5.1 Vital signs

Pulse rate will be measured with automatic device in sitting position after 10 minutes of rest.

12.5.2 Visual Analogue Scale

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 15 and 60 minutes, respectively, after study product administration (as a proxy for systemic uptake). See [Appendix 18.3](#).

12.5.3 Collection of pouches and analysis

Pouches for the determination of nicotine and flavors after administration of the Investigational Product will be collected according to the instructions in the separate dosing instructions document at the pre-specified time-points (see [Table 1 Overall Schedule of Events](#) in Section 9.1). The following time windows will apply for the pouch sampling:

- $\pm 1,5$ min for all time-points.
- 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 and flavor 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.

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.

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'.

12.6.1.2 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:

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

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

[REDACTED] 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/FLAVORS

13.1 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 analyses of nicotine and flavor components. Unused pouches are collected and frozen (-20°C) for analysis and serve as

references in the calculations of extracted doses. Frozen samples of used pouches collected for nicotine determinations will be shipped to Swedish Match laboratories.

13.2 Analysis of nicotine and flavors in pouches

Nicotine and flavor components in used and unused pouches will be analyzed at the Swedish Match laboratories. The analytes will be analyzed simultaneously from the same sample 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 [Appendix 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.

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 July 2017. Performance of clinical part of study is expected to be finished before the end of 2017.

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) which 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 two reference products.

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

- Test articles:**
- 1= ZYN® Smooth containing 3 mg nicotine per portion - 15 min administration.
 - 2= ZYN® Smooth containing 3 mg nicotine per portion - 60 min administration.
 - 3= ZYN® Smooth containing 6 mg nicotine per portion - 15 min administration.
 - 4= ZYN® Smooth containing 6 mg nicotine per portion - 60 min administration.
 - 5= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 15 min administration.
 - 6= ZYN® Smooth containing 3 mg nicotine per portion (alternative manufacturing process) - 60 min administration.
 - 7= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 15 min administration.
 - 8= ZYN® Smooth containing 6 mg nicotine per portion (alternative manufacturing process) - 60 min administration.
 - 9= ZYN® Wintergreen containing 3 mg nicotine per portion (Nicotine analysis) - 60 min administration.
 - 10= ZYN® Wintergreen containing 3 mg nicotine per portion (Flavor analysis) - 60 min administration
 - 11= ZYN® Peppermint containing 3 mg nicotine per portion - 60 min administration.
 - 12= ZYN® Spearmint containing 3 mg nicotine per portion - 60 min administration
- Reference articles:**
- 13= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) (15 min administration).
 - 14= Swedish portion snus PSWL 1.0 g (8 mg nicotine/g) (60 min administration).

17.2 Determination of sample size

The primary endpoint is nicotine extraction. The study will include 20 subjects. A previous study [Lunell E & Curvall M 2011] has informed 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 extraction of the 3 and 6mg 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 endpoints

To compare the estimated in-vivo extracted dose 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. The extracted dose of nicotine from each portion, will be calculated by subtracting the residual amount after use from the mean of 10 unused portions.

17.5.1 Calculation of extracted amount of nicotine

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.

17.5.2 Calculation extracted amount of Flavors

The mean \pm SD extracted dose of each from each pouch, will be calculated.

17.5.3 Pulse rate

Pulse rate will be summarized by treatment and period using descriptive statistics.

17.5.4 Adverse events

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.

17.6 Analysis of secondary endpoints

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.

The intra subject difference of flavor extraction 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

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18.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil_2013.pdf

18.3 Visual Analogue Scale

Intryck av styrka totalt sett (kick)
Overall "product strength"
(head rush, "buzz", feeling alert)

Inget alls "not at all" **Mesta tänkbara "extremely"**

Sätt kryss på linjen motsvarande hur Du upplever det just nu!

19 REFERENCES

1. Fant RV, Henningfield JE, Nelson RA and Pickworth WB. Pharmacokinetics and pharmacodynamics of moist of snuff in humans. *Tob. Control* 1999;8;387-392.
2. Molander L and Lunell E. Pharmacokinetic investigation of a nicotine sublingual tablet. *Eur J Clin Pharmacol.* 2001;56:813-819.
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.