Clinical Study Protocol	
Investigational Medicinal Product	ZYN®
Study Code	SM 17-02
Protocol Version and Date	Final 1.0, 14 Sep 2017

STUDY TITLE

Open observational study of oral health associated with use of a non-tobacco based nicotine pouch (ZYN®) among current daily snus users.

Design	Part 1: Open, randomized, four-way crossover, single administration trial Part 2: Open, observational, follow-up study during 6 weeks
Test product and dosage	Part 1: 1) ZYN® Smooth 3 mg, 2) ZYN® Peppermint 3 mg, 3) 10% sucrose, 4) 10% xylitol. Part 2: Ad libitum use of a ZYN® nicotine pouch, participants can choose from 3 or 6 mg pouches of ZYN® Smooth, ZYN® Peppermint or ZYN® Cinnamon.
Duration of treatment	Part 1: Single administration Part 2: 6 weeks
Sponsor signatory	[redacted] Swedish Match SE-Box 17037 104 62 Stockholm Sweden Maria Skolgata 83 SE-118 85 Stockholm Sweden [redacted]

Principal Investigator

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Clinical study management

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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

Open observational study of oral health associated with use of a non-tobacco based nicotine pouch (ZYN®) among current daily snus users.

Study code	
SM 17-02	
Study period Estimated date of first subject/patient enrolled: Q4 2017 Estimated date of last subject/patient completed: Q1 2018	

Principal Investigator

Prof. Peter Lingström, D.D.S., Ph. D.

Institute of Odontology, Gothenburg, Sweden

Study design

Part 1: Open, randomized, four-way crossover, single administration trial

Part 2: Open, observational, follow-up study for 6 weeks

Objectives

Primary objective(s)

Part 1:

- Assessment of dental plaque acidogenicity after short-term exposure (60 mins) to a nicotine pouch.

Part 2:

- Assessment of dental plaque acidogenicity after 6 weeks of use of a nicotine pouch.

Secondary objective(s)

Part 1:

- Adverse events

Part 2:

- Adverse events
- Changes in the oral microflora
- Occurrence/severity of oral mucosal lesions

Number of subjects/patients planned

Part 1: 20 participants (with available data on all four test articles)

Part 2: 60 participants

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%), normal stimulated salivary secretion rate (≥0.7 ml/min).

Pregnant women, subjects who have a history of hypertension or any cardiovascular disease, subjects with allergy toward composite materials, and those with a history of use of antibiotics during or within the last 4 weeks prior to the study are excluded from participation.

Methodology

For both parts, potential participants will initially be screened for study eligibility and informed consent procedures. Participants in Part 1 are eligible to later on also participate in Part 2 after a new screening visit.

After informed consent, participants will undergo a routine dental and oral examination, assessment of saliva buffer capacity, number of mutans streptococci and lactobacilli.

The subjects in both parts of the study will refrain from approximal tooth cleaning during 72 hours prior to visit and toothbrushing during the last 48 hours prior to visit. They will not eat or drink anything during the last 2 hours prior to visit.

Part 1: Participants will come to the clinic on a total of 4 occasions for testing of the 4 test articles (in randomized order). Each visit is estimated to take 75 min.
Part 2: Participants will participate in a 6-week observational study during which they are encouraged to substitute as much as possible of their snus with the ZYN® test articles. Participants can choose ad libitum from ZYN® Smooth 3 mg or 6 mg nicotine, ZYN® Peppermint 3mg or 6 mg nicotine, or ZYN® Cinnamon 3 mg or 6 mg nicotine pouches. Use of ZYN® products, snus, or any other nicotine delivery product will be monitored, based on self-reports.

Clinical visits are scheduled at screening, after 2 weeks, after 4 weeks, and after 6 weeks. At each visit data will be collected on product use since last visit, AEs, plaque acidogenicity, oral microflora, plaque amount, and oral lesions. Clinical photos will be taken to facilitate comparisons. “Snus lesions” at the site where participants typically place their snus/ ZYN® pouch will be assessed using a 4-degree scale as proposed by Axéll et al (1976). At each visit the subject will report any local and general adverse symptoms.

Investigational products, dosage and mode of administration

Part 1: 1) ZYN® Smooth 3 mg, 2) ZYN® Peppermint 3 mg, 3) 10% sucrose, 4) 10% xylitol.

Part 2: Ad libitum use of a ZYN® nicotine pouch, participants can choose from 3 or 6 mg pouches of ZYN® Smooth, ZYN® Peppermint or ZYN® Cinnamon.

Duration of treatment

Part 1: Single administration (60 minutes)

Part 2: 6 weeks

Duration of subjects involvement in the study

Part 1: Approximately 35 days (single visits)

Part 2: Approximately Six weeks.

Efficacy assessments

Part 1: Dental plaque acidogenicity.

Part 2:

- Adverse Events (AEs) assessed at 2, 4 and 6 weeks
- Biofilm acidogenicity at 2, 4 and 6 weeks compared to baseline
- Changes compared to screening in the oral microflora at 2, 4 and 6 weeks
- Plaque amount at 2, 4 and 6 weeks compared to screening
- Appearance and number of oral mucosal lesions (including presence and grade of "snus lesions" at the site where the pouches typically are placed by the consumer), comparisons will be made with screening findings

Safety assessments

Adverse Events will be recorded at all visits, with particular focus on local irritation at the site of the oral mucosa where the pouch is placed, and in the throat, hiccups and heartburn.

Statistical methods

Part 1: Based on previous experience with the described methodology, a total of 20 subjects will be enough to reliably detect a clinically significant increased plaque acidogenicity with the pouched products versus the negative control.

Part 2: A 6 week observation period is reasonable to assess putative changes in the oral mucosa resulting from use of the nicotine pouches given that "snus lesions" among habitual snus users regress within a few weeks after cessation of exposure. A 6 week observation period is also supported by the fact that the other measures of oral health to be assessed (biofilm acidogenicity, oral microflora, plaque amount) are known to potentially change within a few weeks. With an estimated dropout rate of 25% a total of 45 fully evaluable subjects are expected with a total inclusion of 60 subjects. Descriptive statistics will be used for reporting the results of monitoring of the oral mucosa and for subjective adverse symptoms.

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4 LIST OF ABBREVIATIONS AND DEFINTIONS OF TERMS

Abbreviation or term	Explanation
ADL	Activities of daily living
AE	Adverse event
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration time curve
BP	Blood pressure
BMI	Body mass index
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
IMP	Investigational medicinal product
MedDRA	Medical dictionary for regulatory activities
min	minute
MPA	Medical products agency
N	number
NCA	Non-compartmental analysis
NIH	National Institute of Health
NOAEL	No observed adverse effect level
NRT	nicotine replacement therapy

OTC	Over the counter
SAR	Serious adverse reaction
PPAS	Per protocol analysis set
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
WHO	World Health Organisation

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 subjects are well familiar to Swedish snus. In cases of accidental overdose, standard supportive measures should be adopted as required and reported according to section 5.1.

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/patient's medical records.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor

Swedish Match
SE-Box 17037
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Sweden
Maria Skolgata 83
SE-118 85 Stockholm, Sweden

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Principal Investigator

Prof. Peter Lingström, D.D.S., Ph. D.

[redacted]

Clinical conduct and management
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Dag Hammarskjölds väg 13
SE-752 837 Uppsala, Sweden

[redacted]

[redacted]

6.1.1 Investigational Product manufacturing and packaging

The investigational products will be manufactured and packaged compliant with Swedish law on food production.

6.1.2 Identity of investigational products

For Part 1 the investigational product will be delivered by Swedish Match to the laboratory in identical containers labelled with unique identification numbers in accordance with the randomization list.

For Part 2 the investigational product will be delivered in its original container, packaged in a secondary packaging containing five cans. The container will be labeled in English. The secondary packaging will be labeled in Swedish. Signatures required are provided in Appendix 18.1.

7 INTRODUCTION

7.1 Project background

Clinical experience does not indicate that habitual use of regular, tobacco based snus affects biofilm acidogenicity. Contributing factors may be that snus includes food approved pH regulating substances (such as sodium carbonate) which maintains a relatively high pH in the snus pinch/pouch (c. pH 8-8.5), nicotine itself does not seem to affect biofilm acidogenicity, and that the tobacco does not function as a substrate for the oral microflora. These circumstances may help to explain why caries does not seem to be more prevalent among snus users than among non-tobacco users. The non-tobacco based nicotine pouch (ZYN®) is an alternative form of orally delivered nicotine. The physical properties of ZYN® in terms of pH is the same as with regular, tobacco-based snus and the product is used the same way, that is, it is placed in the upper sulcus for 30-60 minutes. However, the matrix for the nicotine in ZYN® is different from that in regular snus: microcrystals of maltitol and cellulose instead of ground tobacco leaves. In food stuffs, maltitol and cellulose have not been associated with changes in biofilm acidogenicity. However, the prolonged exposure (c. 30-60 minutes) associated with use in a product like ZYN® constitutes a somewhat different type of exposure. Therefore, although there are no priori reasons to believe that use of ZYN® will adversely affect biofilm acidogenicity, it is reasonable to rigorously assess this possibility in the context of a controlled clinical trial. Particularly since ZYN® is marketed as a consumer product and therefore is used ad libitum by consumers.

Smokers frequently exhibit oral mucosal lesions which can be located anywhere in the oral mucosa, such as leukoedema, smoker's palate, smokers's melanosis, lingua villosa/nigra, leukoplakia and erythroplakia[1]. It is generally assumed that the main reason for these lesions is the smoker's exposure to the combustion products in tobacco smoke, most of which are found in the tar particles in the smoke. Regular snus users may develop mucosal lesions, "snus lesions" (SILs) in the upper sulcus at the site where they typically place the snus pinch/pouch, however, to a significantly lesser extent than among smokers[2]. The biology of these lesions is clearly different from most of the mucosal lesions associated with smoking: they are strictly localized to the site of exposure in the upper sulcus, they are reversible within weeks after cessation of exposure or if the snus user changes the location of the snus pouch, and they do not appear to be pre-malignant. The exact mechanism behind these lesions remains unclear. It has been suggested that the high pH of snus could result in a localized, chemical irritation of the mucosa. However, the observation that snus lesions are much less prevalent among users of pouched snus compared to loose snus suggests that physical irritation from the tobacco particles in snus may also play a role. The nicotine in snus may be another significant factor. For instance, mucosal lesions have been observed with lozenges of pharmaceutical nicotine replacement therapy. A recent study assessed the oral safety of a sublingual tablet containing 2 mg nicotine with regard to lesions at the site of application[3]. In a prospective follow-up of smokers using the sublingual nicotine tablet over

a period of 3-6 months 8/30 subjects displayed lesions in the floor of the mouth during the 6-month medication period, all of which appeared in the first 1-6 weeks. By the 6-month visit all such lesions had resolved[3].

The physical properties of ZYN® in terms of pH is the same as with regular, tobacco-based snus (pH c. 8-8.5) and it is used the same way as snus, that is, it is placed in the upper sulcus for 30-60 minutes. Because of these circumstances it is unclear to what extent use of ZYN® may cause similar mucosal lesions as regular, tobacco based snus. The comparable pH and the nicotine delivery may indicate a similar potential, but the absence of tobacco particles may result in less physical irritation.

The main aim of the present study is to assess the safety and tolerability of the non-tobacco based nicotine pouch (ZYN®), particularly with regard to its potential to adversely affect biofilm acidogenicity. A secondary aim is to investigate to which extent ZYN® has the potential to produce mucosal lesions at the site of application in the oral cavity similar to those occasionally observed among regular snus users and whether pre-existing snus lesions among the included subjects may improve or resolve during a 6 week observation period during which the participants substitute their regular snus with ZYN® products.

Adverse Events will be recorded at all visits, with particular focus on local irritation at the site of the oral mucosa where the pouch is placed, or in the throat, and hiccups and heartburn (which are common side effects with all types of nicotine exposure).

7.2 Investigational medicinal product

7.2.1 Product characteristics

The Non-tobacco based nicotine pouch (ZYN®) will be delivered in identical glass vials for Part 1 and in its original container for Part 2.

Part: 1) ZYN® Smooth 3 mg, 2) ZYN® Peppermint 3 mg, 3) 10% sucrose solution, 4) 10% xylitol solution.

Part: 2: Ad libitum use of a ZYN® nicotine pouch, participants can choose from 3 or 6 mg pouches of ZYN® Smooth, ZYN® Peppermint and ZYN® Cinnamon.

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. The assessments of plaque acidogenicity require participants to refrain from brushing their teeth for 48 hours prior to the examination, and the procedure involves exposure to a positive control substance (sucrose)

that will lower their plaque pH. This may theoretically have adverse effects on the participants' dental health, but previous studies have shown no such clinical adverse effects after refraining from toothbrushing for such a short period of time.

All mentioned potentially adverse effects of study participation are likely to be minor and clinically insignificant. As to the nicotine exposure, 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. Aside from the nicotine, all ingredients used in the test products are food-approved (similar to ingredients in snus).

Pregnant women or individuals with a history of hypertension or any other cardiovascular disease who may be particularly vulnerable to nicotine exposure are excluded from participation. Individuals with a lower than average saliva production are also excluded as adverse effects related to xerostomia may adversely influence outcomes and thus bias the results (it is expected that few if any of the included participants will have problems related to xerostomia as they are all regular snus users since more than one year).

The procedures used to assess oral health, including measurements of plaque acidogenicity, are standard procedures used at odontological research facilities and are not associated with any major discomfort or significant adverse events. The procedures are unlikely to adversely affect the participants' oral health in the long-term because of their limited duration. In fact, theoretically, participation in the studies may help to improve participants' long-term oral health through an increased awareness of the significance of dental hygiene. The studies will not involve invasive procedures.

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

7.3.1 Summary of risk management

Subjects will for Part 1 remain in the research clinic during the administration of the Investigational Product and will be closely monitored by medical staff.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective(s)

The primary objective of the present study is to evaluate the amount of dental plaque acidogenicity from the non-tobacco based nicotine pouch.

Part 1: Assessment of dental plaque acidogenicity after short-term exposure (60 mins) to study products (a flavored and an unflavored brand of the nicotine pouch), 10% sucrose (positive control), and 10% xylitol (negative control).

Part 2: Assessment of dental plaque acidogenicity during a total of 6 weeks of ad libitum use of the nicotine pouch.

8.2 Primary endpoint

Part 1:

Assessment of dental plaque acidogenicity after short-term exposure (60 mins) of nicotine pouch.

Part 2:

Assessment of dental plaque acidogenicity after 6 weeks of use of nicotine pouch.

8.3 Secondary objectives

The secondary objective of the present study is to evaluate the clinical tolerability and safety of of ZYN® with respect to effects on the oral mucosa

8.4 Secondary endpoints

The secondary endpoints will be

- Adverse events (part 1 and part 2)
- Changes in the oral microflora (part 2)
- Oral mucosal lesions (part 2)

9 INVESTIGATIONAL PLAN

9.1 Overall study design and schedule of events

Part 1 of the study is an open, randomized, four-way crossover, single administration for 60 minutes (measured at 0 minutes and then at 2, 5, 10, 20, 30, 40, 50 and 60 minutes). Subjects will be randomized to one of four treatment sequences using a flavored and an unflavored brand of the nicotine pouch), 10% sucrose (positive control), and 10% xylitol (negative control) with one-week washout. The number of healthy subjects aged over 19 needed to be evaluate should complete the four-period cross over is estimated to be 20. Each visit lasts for about 75 min.

Table 1 Schedule of events Part 1

EVENT	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Follow-Up
	Screening Day -10 - -3	**Day 0**	**Day 7(+2)**	**Day 14(+2)**	**Day 21(+2)**	**Day 28(+/-2)**
ELIGIBILITY CHECK	✓					
HEALTH CHECK (Physical exam)	✓					
MEDICAL HISTORY	✓					
CONCOMITANT MEDICATION	✓	✓	✓	✓	✓	✓
INFORMED CONSENT	✓					
URINE PREGNANCY TEST	✓					
DOSAGE OF STUDYPRODUCT		✓	✓	✓	✓	
AE INTERVIEW	✓	✓	✓	✓	✓	✓
DENTAL PLAQUE ACIDOGENICITY		✓	✓	✓	✓	

Part 2 of the study is an open, observational, safety and tolerability study during 6 weeks. The subjects will use the non-tobacco based nicotine pouch (ZYN®) *ad libitum*. At each visit the subject will report any local and general adverse symptoms.

All subjects are seen on an individual basis and each visit lasts for about 90 min.

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%), normal stimulated salivary secretion rate (≥0.7 ml/min).

Table 2 Schedule of events Part 2

EVENT	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Follow-up
	Screening Day -2	**Day 0**	**Day 14 (+/-2)**	**Day 28 (+/-3)**	**Day 42 (+/-4)**	**Day 49 (+/-2)**
ELIGIBILITY CHECK	✓					
HEALTH CHECK (Physical exam)	✓					
MEDICAL HISTORY	✓					
CONCOMITANT MEDICATION	✓	✓	✓	✓	✓	✓
INFORMED CONSENT	✓					
URINE PREGNANCY TEST	✓					
AE INTERVIEW	✓	✓	✓	✓	✓	✓
PHOTO	✓		✓	✓	✓	
INSPECTION OF THE ORAL CAVITY (MUCOSAL LEISIONS)	✓		✓	✓	✓	
DENTAL PLAQUE ACIDOGENICITY	✓		✓	✓	✓	
ORAL MICROFLORA	✓		✓	✓	✓	
PLAQUE AMOUNT	✓		✓	✓	✓	
DIARY FOR COMPLICANCE			✓	✓	✓	
SUPPLY OF STUDY PRODUCT	✓	✓	✓	✓		

9.2 Rationale for study design and dose groups

The rationale for the choice of the study design is from a recent study that assessed the oral safety of a sublingual tablet containing 2 mg nicotine with regard to lesions at the site of application[3] and from a prospective follow-up of smokers using the sublingual nicotine tablet over a period of 3-6 months 8/30 subjects displayed lesions in the floor of the mouth during the 6-month medication period, all of which appeared in the first 1-6 weeks. By the 6-month visit all such lesions had resolved[3].

10 STUDY POPULATION

10.1 Recruitment

Healthy adult males and females will be recruited using an advertisement in the local newspaper. Following a telephone interview to evaluate eligibility, potential participants are invited to a screening visit. The potential participants will then submit a Health Declaration that will be checked by the responsible Investigator.

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 screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

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

If a subject cannot receive the planned dose of investigational product within 28 days after screening (*i.e.*, the time interval between signing informed consent until dose administration) the subject should be rescreened before proceeding in the trial.

10.3 Number of subjects

Part 1: 20 participants (with available data on all four test articles)

Part 2: 60 participants

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 $\geq$1 year.
2. Consent to participate voluntarily and sign Informed Consent Form prior to any study procedure.
3. Healthy male/female, age $\geq$19. Female subjects should have a negative pregnancy test.
4. Willing and able to comply with study procedures.
5. Normal stimulated salivary secretion rate ($\geq$0.7 ml/min).

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 nicotine.
4. Subjects who are pregnant.
5. Allergy towards composite materials.
6. Antibiotic use during or within the last 4 weeks prior to the study period.

10.6 Restrictions during the study

The subjects in both parts of the study will refrain from approximal tooth cleaning during the 72 hours prior to visit and toothbrushing during the last 48 hours prior to visit. They will not eat or drink anything during the last 2 hours prior to visit.

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 will not be replaced during the course of the study.

10.8 Randomization

For Part 1 the 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

Part 1: 1) ZYN® Smooth 3 mg, 2) ZYN® Peppermint 3 mg, 3) 10% sucrose solution, 4) 10% xylitol solution.

Part 2: Ad libitum use of a ZYN® nicotine pouch, participants can choose from 3 or 6 mg pouches of ZYN® Smooth, ZYN® Peppermint or ZYN® Cinnamon.

11.2 Packaging and labelling

Part1:

The Non-tobacco based nicotine pouch (ZYN®) will be delivered by Swedish Match to the laboratory in identical containers labelled with unique identification numbers in accordance with the randomization list.

The positive- and negative control will be prepared by the laboratory and labelled with unique identification numbers by the laboratory in accordance with the randomization list.

The container will be labelled with unique identificaton numbers (in Swedish):

Trial code: SM 17-02
Subject No.: 1 (20)
Visit:
Dose:
Storage conditions: Refrigerated storage

Part2:

The Non-tobacco based nicotine pouch (ZYN®) will be delivered in its original container. Labeling of the secondary packaging will be in Swedish.

The secondary packaging will be labelled by the laboratory (in Swedish):

Non-tobacco based nicotine pouch (ZYN® Smooth 3 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

Non-tobacco based nicotine pouch (ZYN® Smooth 6 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

Non-tobacco based nicotine pouch (ZYN® Peppermint 3 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

Non-tobacco based nicotine pouch (ZYN® Peppermint 6 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

Non-tobacco based nicotine pouch (ZYN® Cinnamon 3 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

Non-tobacco based nicotine pouch (ZYN® Cinnamon 6 mg).
For clinical trial.
Trial code: SM 17-02
Subject No.: 1 (60)
Batch No.:
Expiry date:
Dosage: Ad libitum
Responsible investigator: Prof. Peter Lingström, D.D.S., Ph. D.
Keep out of reach of children.

11.3 Conditions for storage

The Investigational Product will be stored in the access-controlled cold (ca 7°C) storage area at the investigational site, as per storage conditions specified by the Sponsor.

11.4 Dispensing and accountability

Part 1:

The study product will be dispensed as per randomisation schedule by site personnel.

Part 2:

The subject will try out the appropriate product during two days and after that collect 14 days supply of the selected product on the visits to the clinic. Subjects are allowed to switch between all of the study products ad libitum.

The Investigator will maintain a *Product Dispensing Log* based on the amount of cans detailing the dates and quantities of study product received, dispensed to and used by each subject/patient and study product returned or destroyed at the end of the study. Any discrepancies between dispensed and returned investigational products 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

Part 1:

A single dose will be given on each study day.

Part 2:

The subjects will be recommended to replace as many as possible of their regular snus products (ideally all) with ZYN® products during the 6 week period and to use as much ZYN® as they need.

The duration of the treatment period is 6 weeks. It is entirely at the discretion of the subject when he/she will use the pouch during the day and how many pouches per day that are used.

11.6 Treatment compliance

Part 1:

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

Part 2:

In part 2 of the study the subjects will receive enough study products for 14 days period.

The amount of study product for part 2 will be recorded in a paper diary.

11.7 Return and destruction of investigational products

Any unused study product will be collected and kept at site until returned to the Sponsor. 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 destroyed 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 (Overall study design and schedule of events).

12.1 Part 1

Screening

Subjects will be seen in a screening visit (day -10 to -3), during which informed consent will be obtained prior to any study procedures. The past medical /surgical history will be reviewed. Information will be obtained regarding any current medications. Health check and saliva secretion assessment will be completed. Urine sample for pregnancy test (female only) will be collected.

Visit 2, 3, 4 and 5:
Eligible subjects will return for their first dosing visit on visit 2, 3, 4 and 5. They will be interviewed regarding concomitant medication and AEs (Basal events) since the screening visit. After that assessment of dental plaque acidogenicity and plaque amount will be performed. After the initial assessments the subject will be given the study product, positive or negative control. Study products will be kept in the vestibule during the 60 minutes of acidogenicity assessment while positive and negative control will be rinsed in the mouth for 60 seconds. After that dental plaque acidogenicity will be assessed 8 more times during 60 minutes followed by an assessment of plaque amount.

Table 3 Detailed schedule of events Part 1

TIME POINT	PRE-DOSE	0 MIN	1 MIN	2 MIN	5 MIN	10 MIN	20 MIN	30 MIN	40 MIN	50 MIN	60 MIN	65 MIN
Con. Med	X											
AE interview	X											X
Plaque Acidogenicity		X		X	X	X	X	X	X	X	X	
Dosage of pouch		X										
Removal of pouch											X	
Pos/Neg control in		X										
Pos/neg control out			X									

Follow-up:
A phone call will be made by the study researcher to the subjects 5-10 days after end of visit 5. AEs and concomitant medication will be checked.

12.2 Part 2

Screening:
Subjects will be seen in a screening visit (day -2), during which informed consent will be obtained prior to any study procedures. The past medical/surgical history will be reviewed. Information will be obtained regarding any current medications. Health check and saliva secretion assessment will be completed. Urine sample for pregnancy test (female only) will be collected.

Assessment off the following parameters will be performed:

- Inspection of the oral cavity
- Photo
- Biofilm acidogenicity
- Oral microflora
- Plaque amount

At the end of the screening visit the subject will receive samples of the study products, 3 or 6 mg pouches of ZYN® Smooth, ZYN® Peppermint or ZYN® Cinnamon to be able to choose based on their preferences.

Visit 2

Two days after the screening visit the subject will return to the clinic and pick up a supply for the first two weeks. Subsequent, the study product will be supplied on the forthcoming clinic visits.

Visit 3, 4 and 5:

Eligible subjects will return for their visit 3, 4 and 5. They will be interviewed regarding concomitant medication and AEs (Basal events) since the last visit.
The following assessments will performed:

- Inspection of the oral cavity
- Photo
- Biofilm acidogenicity
- Oral microflora
- Plaque amount

Follow-up:

A phone call will be made by the study researcher to the subjects 5-10 days after end of visit 4. AEs and concomitant medication will be checked.

12.3 Recording of data

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

12.4 Demographics and other baseline characteristics (Part 1 and Part 2)

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 to verify that the eligibility criteria are met.

12.4.4 Physical examination

Physical examination will be obtained by interview to verify that the eligibility criteria are met.

12.4.5 Prior and concomitant medication

Prior medication will be obtained by interview in order to verify that the eligibility criteria are met (see also Section10.6).

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.

12.4.6 Pregnancy urine test

Pregnancy urine test will be performed at screening visit (females only) at the research clinic using dip sticks.

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 study 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 Examination of the oral cavity

Clinical examination of the oral cavity will be performed at screening and visit 3, 4 and 5.

The oral mucosa will be inspected and any pathological changes will be recorded and classified. *Lesions* in the mucosa at the placement of the pouch, particularly "snus lesions" (SILs), will be registered according to four grade clinical scale suggested by Axéll et al[1]. In addition gingival retractions will be recorded

Clinical examination of the oral cavity will be performed according to Axéll et al (1976)[1]:

- **Degree 1**. A superficial lesion with a color similar to the surrounding mucosa and with slight wrinkling. No obvious mucosal thickening.
- **Degree 2**. A superficial, whitish or yellowish lesion with wrinkling. No obvious thickening.
- **Degree 3**. A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal colors, obvious thickening.
- **Degree 4**. A marked yellowish to brown and heavily wrinkled lesion with intervening deep reddened furrows and/or heavy thickening.

By the 6-week visit (study termination) it will be summarized to which extent such lesions have changed (according to the above scale or have resolved completely).

Oral *leukoplakias* and *erythroplakias* will be followed up according to standard clinical routines.

Local symptoms reported by the subject, are recorded at each visit, elicited using open-ended general questions. Other adverse events reported spontaneously are also recorded and duration thereof.

The oral cavity will be documented by photography.

12.5.2 Dental plaque acidogenicity

Plaque acidogenicity will be measured using the microtouch method. An iridium microelectrode (Beetrode MEPH-1, WPI Instruments, New Haven, Conn., USA) will be inserted into the plaque on two buccal surfaces in the upper jaw and two approximal surfaces in the upper and lower jaw. The electrode will be connected to an Orion SA720 pH/ISE Meter (Orion Research, Boston, Mass., USA) to which also a reference electrode is connected. The reference electrode is placed into a solution of 3 M KCl to which also a finger of the volunteer is placed in order to create a salt bridge. Prior to and during each test session, the electrode is calibrated against a standard buffer at pH 7.00. After baseline registration (0 min), the subjects will rinse with the sucrose or xylitol solution or use the study products for 1 min after which pH was measured at 8 different time points up to 60 min.

12.5.3 Plaque amount

The plaque amount will be assessed with a plaque score calculated by the index described by Silness and Löe (1964)[6]. The amount of plaque will be measured on all surfaces. For each tooth six sites (mesio-buccal, buccal, disto- buccal, disto-lingual, lingual and mesio-lingual) from score 0-3.

12.5.4 Oral microflora

Pooled plaque samples will be collected by a sterile toothpick according to Kristoffersson and Bratthall (1982)[7] from the buccal areas of respective quadrants.

12.5.5 Saliva sampling

The salivary factors pH and flow rate (unstimulated and stimulated) will be measured. Saliva will be collected into a beaker and the secretion rate calculated in ml/min. The buffer capacity will be determined using a chairside kit (CRT Buffer®, Vivadent, Germany)

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:

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.

13 PROCEDURES FOR BIOLOGICAL SAMPLES

13.1 Sample collection for pharmacokinetic measurements

No blood samples will be collected in this study.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The trial 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. The trial will be consistent with Good Clinical Practice (GCP) and applicable regulatory requirements.

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

14.2 Ethics and regulatory review

The Principal Investigator is responsible for submission of the CSP, the patient information and ICF, any other written information to be provided to the subjects/patients and any advertisements used for recruitment of subjects/patients to applicable Independent Ethics Committee (IEC) for approval.

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

The Sponsor will provide IEC and Principal Investigators with safety updates/reports according to local requirements. Progress reports and notifications of SUSARs will be provided to the IEC according to local regulations and guidelines.

It is the responsibility of the investigator to obtain approval of the trial protocol/amendments from EC. The investigator should file all correspondence with the EC. Copies of EC approvals should be forwarded to CTC AB.

14.3 Subject/Patient information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject/patient (or the subject's/patient's legally acceptable representative and/or witness, as applicable) adequate verbal and written information before any study specific assessments are performed.

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

Before performing any study-related procedures the ICF must be signed and personally dated by the subject/ patient (or their legally acceptable representative and/or witness, as applicable) and by the Investigator. A copy of the subject/patient information including the signed ICF will be provided to the subject/patient.

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

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

14.4 Subject/Patient information card

The subject/patient will be provided with a Subject/Patient information card including the following information:

- That he/she is participating in a clinical study
- Subject study ID
- That he/she is treated with the IMP
- The name and phone number of the Investigator

- Name and address of the Sponsor

14.5 Subject/Patient data protection

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

The potential study subject/patient (or the subject's/patient's legally acceptable representative and/or witness, as applicable) should be informed that by signing the ICF he/she approves that authorized representatives from Sponsor and CTC, the concerned IEC and CA have direct access to his/her medical records for verification of clinical study procedures. This agreement is to be substantiated in a separate document, according to local requirements.

The subject/patient has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete.

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

14.6 Changes to the approved clinical study protocol

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

14.7 Audits and inspections

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

14.8 Insurance

Subjects will be covered under the Sponsors liability insurance policy through If. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects/patients are also protected in accordance with national regulations, as applicable. Göteborgs university has a company insurance covering services performed at the laboratory.

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) utilised.

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

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

15.2 Clinical monitoring

The study site will be periodically visited by a Monitor from an independent group at times agreed on by the Investigator and the Monitor. At the time of each monitoring visit, the function of the Monitor is to:

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

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

Monitoring visits to the trial site will be made periodically during the trial, to ensure that all aspects of the protocol are followed. The report will be reviewed for verification of agreement with data on Case Report Forms. The investigator/institution guarantee access to CRFs and report and all relevant documents by CTC AB and appropriate regulatory agencies.

The trial site may also be subject to quality assurance audit by CTC AB as well as inspection by appropriate regulatory agencies.

It is important that the investigator and their 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

A separate *Source Data Verification List* will be generated for each site 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, CAs and the IECs, if required.

15.4 Study agreements

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

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

15.5 Study time table and end of study

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

The study is expected to start in Quarter 4, 2017 and to be completed by Quarter 2, 2018.

15.6 Discontinuation of the study

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

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

15.7 Reporting and publication

15.7.1 Clinical study report

A summarising report must be submitted to the applicable IEC within 12 months after completion of the study (in accordance with LVFS 2011:19, Chapter 9).

A clinical study report (CSR), in compliance with ICH E3; *Structure and content of clinical study reports*, describing the conduct of the study 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. The study results will be reported in the EudraCT database per applicable regulations within 12 months after completion of the study.

15.7.2 Annual safety report

If the study duration exceeds one year, the Sponsor must submit an annual safety report to the CA and to the IEC. The report shall summarize all SAEs and contain an update of the risk-benefit evaluation if there has been any change since the approval of the clinical study.

15.7.3 Confidentiality and ownership of study data

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

15.7.4 Publication

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

15.8 Archiving

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

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

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

16 DATA MANAGEMENT

16.1 Case report form

Data will be collected in paper CRFs specifically designed for this study. The Investigator or an authorised 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 sign off the CRF at each visit and 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 initialled and dated. Correction fluid must not be used. Only persons authorised by the Investigator are allowed to make entries to the CRF.

A Case Report Form (CRF) is required and should be completed for each included subject. The completed original CRFs forms as templates are the sole property of CTC AB and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from CTC AB.

16.2 Database management plan and database design

Detailed information on data management will be described in a study-specific Data Management Plan (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 computerised edit checks and queries for identifying data values that are outside the allowed range, protocol violations, incomplete or inconsistent. The Data Validation Plan 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 performed 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 World Health Organisation (WHO) Anatomic Therapeutic Chemical (ATC) classification system.

All coding will be approved by Sponsor.

16.5 Database lock

When all data have been entered and discrepancies solved, the database will be locked and the data will be analysed. The data cleaning process will be performed in close collaboration between the Sponsor and CTC.

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

Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), median, 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.

Descriptive statistics will be used for the reporting of the results.

17.2 Determination of sample size

Part 1: Based on previous experience with the described methodology, a total of 20 subjects will be enough to reliably detect a clinically significant increased plaque acidogenicity with the pouched products versus the negative control.

Part 2: A 6 week observation period is reasonable to assess putative changes in the oral mucosa resulting from use of the nicotine pouches given that "snus lesions" among habitual snus users regress within a few weeks after cessation of exposure (Wallström et al, [1999][3]). A 6-week observation period is also supported by the fact that the other measures of oral health to be assessed (biofilm acidogenicity, oral microflora, plaque amount) are known to potentially change within a few weeks. With an estimated dropout rate of 25% a total of 45 fully evaluable subjects are expected with a total inclusion of 60 subjects. Descriptive statistics will be used for reporting the results of monitoring of the oral mucosa and for subjective adverse symptoms. Each subject receives six weeks' treatment.

17.3 Analysis data sets

17.3.1 Full analysis set

Part 1:

The Full Analysis Set (FAS) will consist of all subjects who have been randomised and received at least one dose of the product. This population will be used as Safety analysis set.

Part 2:

The Full Analysis Set (FAS) will consist of all enrolled subjects. This population will be used as Safety analysis set.

17.3.2 Per protocol analysis set

Part 1:

The Per Protocol Analysis Set (PPAS) will consist of all subjects who have been randomised and completed the study period without any major protocol deviations. All protocol violations will be judged as major or minor at the clean file meeting.

Part 2:

The Per Protocol Analysis Set (PPAS) will consist of all subjects who have been enrolled and completed the study period without any major protocol deviations. All protocol violations will be judged as major or minor at the clean file meeting.

17.4 Description of study population for both parts

17.4.1 Demographics and baseline characteristics

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

17.4.2 Medical/surgical history and prior/concomitant medication

Medical/surgical history and prior/concomitant medications will be presented by product using descriptive statistics and listings.

17.4.3 Treatment compliance

The number of subjects treated in each treatment period and their product will be tabulated. In Part 2 it will also be tabulated to which extent participants replaced their habitual snus with study products.

17.5 Analysis of primary endpoints

17.5.1 Dental plaque acidogenicity Part 1

Assessment of dental plaque acidogenicity for 60 minutes of use of nicotine pouch will be described using AUC and changes from 0 minutes to 60 minutes and presented using summary statistics.

17.5.2 Dental plaque acidogenicity Part 2

Assessment of dental plaque acidogenicity for 6 weeks of use of nicotine pouch will be described using change from baseline to 6 weeks and presented using summary statistics.

17.6 Analysis of secondary endpoints

17.6.1 Adverse events Part 1 and Part 2

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

17.6.2 Biofilm acidogenicity Part 2

Changes compared to baseline in the oral microflora at 2, 4 and 6 weeks

17.6.3 Changes in oral microflora Part 2

Biofilm acidogenicity at 2, 4 and 6 weeks compared to baseline

17.6.4 Oral mucosal lesions Part 2

Appearance and number of oral mucosal lesions (including presence and grade of "snus lesions" at the site where the pouches typically are placed by the consumer), comparisons will be made with baseline findings

17.7 Statistical/analytical issues

17.7.1 Adjustments for covariates

Not applicable

17.7.2 Handling of dropouts or missing data

No imputations for missing values will be used

17.7.3 Multi-centre studies

Not applicable

17.7.4 Multiple comparison/multiplicity

Not applicable

17.7.5 Examination of subgroups

Not applicable

17.7.6 Interim analyses and data monitoring

Not applicable

18 APPENDICES

18.1 Signature page

[redacted]

[redacted]

[redacted]

[redacted]

18.2 Declaration of Helsinki

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

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