| **Clinical Study Protocol** | |
|---|---|
| Investigational products | ZYN |
| Sponsor study code | SM22-01 |
| Protocol Version and Date | Final v1.1; 09SEP2022 |

# Effects of two types of nicotine pouch (NP) products on oral health, specifically focusing on dental caries, soft tissues, and periodontal health in current, daily tobacco-based snus users transitioning to NPs

**Test products and doses**

ZYN Dry Smooth 6 mg
ZYN Dry Cool Mint 6 mg
ZYN Dry Citrus 6 mg
ZYN Moist Smooth 9 mg
ZYN Moist Cool Mint 9 mg
ZYN Moist Citrus 9 mg

**Sponsor signatory**

[redacted]

Swedish Match North Europe
Maria Skolgata 83
SE-118 53 Stockholm
Sweden

[redacted]

**Principal Investigator**

Peter Lingström, DDS, PhD
Professor, Senior Dental Officer

Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg
Box 450
SE-405 30 Gothenburg
Sweden

[redacted]

**Clinical study conducts and management**

Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg
Box 450
SE-405 30 Gothenburg
Sweden

CTC Clinical Trial Consultants AB
Dag Hammarskjölds väg 10B
SE-752 37 Uppsala
Sweden

# 1 STUDY SYNOPSIS

**Study title**

Effects of two types of nicotine pouch (NP) products on oral health, specifically focusing on dental caries, soft tissues, and periodontal health in current, daily tobacco-based snus users transitioning to NPs

| **Study code** | **Planned study period** |
|---|---|
| SM22-01 | Q4 2022 to Q3 2023 |

**Principal Investigator**

Peter Lingström, DDS, PhD
Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg
Box 450
SE-405 30 Gothenburg
Sweden

**Study design**

This is an open-label, two-armed, randomized, longitudinal study, designed to assess the effects on dental plaque acidogenicity and other oral variables in current, daily tobacco-based snus users who completely substitute their snus use with either ZYN Dry or ZYN Moist nicotine pouches (NPs). The investigational products (IPs) are unflavored (Smooth) or flavored (Cool Mint and Citrus) NPs and of different nicotine strengths (ZYN Dry 6 mg and ZYN Moist 9 mg).

**Objectives**

Primary objective

The primary objective of the study is to compare the impact on dental plaque acidogenicity after 4 weeks of substitution of tobacco-based snus with either ZYN Dry or ZYN Moist.

Secondary objectives

1. To compare the effects on dental plaque acidogenicity following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. To compare the number of cariogenic microorganisms in dental plaque following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
3. To compare the amount of plaque following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
4. To evaluate the degree of oral mucosal lesions following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
5. To compare tooth discoloration following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
6. To evaluate the safety and tolerability of ZYN Dry and ZYN Moist in current, daily tobacco-based snus users.

Exploratory objectives

1. To compare biomarkers of exposure (BoE) in saliva following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. To compare BoE in urine following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.

The results of the exploratory objectives may not be reported in the clinical study report (CSR).

**Endpoints**

Primary endpoint

The difference between ZYN Dry and ZYN Moist in terms of changes in dental plaque acidogenicity, assessed by measuring pH in plaques, after substitution of tobacco-based snus for 4 weeks.

Secondary endpoints

1. The difference in dental plaque acidogenicity, assessed by measuring pH in plaques, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. The difference in the counted number of cariogenic microorganisms in dental plaque following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
3. The difference in the amount of plaque, counted and scored 0-3, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
4. The difference in the degree of oral mucosal lesions, by presence, visual grading, and histopathological evaluation of biopsies, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
5. The difference in tooth discoloration, by means of colorimetry using a colorimeter and by visual shade grading using a shade guide, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
6. Frequency, intensity, and seriousness of adverse events (AEs).

Exploratory endpoints

1. The difference in analyzed BoE in saliva following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. The difference in analyzed BoE in urine following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.

**Number of subjects planned**

Approximately 60 subjects will be screened with the aim of achieving 46 randomized subjects and 40 fully evaluable subjects. A fully evaluable subject is defined as a subject who has used the IPs ad libitum for 4 weeks and who has completed all study visits. An effort will be made to randomize at least 5 female subjects into each treatment arm (each arm consisting of 23 subjects in total).

**Diagnosis and main eligibility criteria**

Healthy male or female subjects aged ≥21 to ≤55 years who have used tobacco-based snus for ≥1 year, with a minimum weekly consumption of 3 or more snus cans, may be eligible for participation in the study. Subjects must be willing to comply with study procedures and give written informed consent.

Subjects who are pregnant, breastfeeding, or intend to become pregnant during the course of the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease or other medical condition, including severe oral conditions (*e.g.*, open caries lesions, severe periodontal health, extensive prosthetic work), that may interfere with the evaluation of the effects of the IPs or may put the subject at risk because of participation in the study, and/or intend to stop using nicotine-containing products, will be excluded from the study.

**Methodology**

Subjects will report to the clinic for a screening visit followed by 4 treatments visits on separate days. The screening (Visit 1) will take place within 5 weeks prior to the first treatment visit and involve an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, and a brief oral examination, urine pregnancy testing (women of childbearing potential only), and collection of medical history. Also, the salivary secretion rate and buffer capacity will be assessed. At the end of the screening, the subjects will be asked about their flavor preferences of IPs.

Before each treatment visits (Visit 2-5; Day -28, Day 0, Day 14, and Day 28, respectively), scheduled at the same time of the day for each subject, the subjects will refrain from approximal tooth cleaning for 48 h and toothbrushing for 24 h prior to the visits. Also, the subjects will be instructed not to eat, drink, chew gum, or any other mouth related procedures 1 h before the visits and during the visits.

On the first treatment visit (Visit 2, Day -28), the subjects will report to the clinic for a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), and sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva for assessment of BoE. Data regarding plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and followed for 60 min. Oral hygiene and dietary habits will also be assessed by a questionnaire. Moreover, the subjects will be provided with a standard toothpaste containing fluoride (sodium fluoride-containing toothpaste; 1450 ppm F) that must be used by all subjects throughout the whole study. The subjects will use their regular toothbrush, which may be manual or electric, during the whole study. Finally, the teeth will be cleaned with rotating rubber cup and polishing paste. The subjects will electronically register the number of tobacco-based snus pouches used per day, the timepoints, and the estimated usage time for four weeks (to Visit 3, Day 0).

On the second treatment visit (Visit 3, Day 0), a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), and sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva and urine for assessment of BoE will be performed. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and followed for 60 min. A biopsy of the oral mucosa will be collected. Finally, the teeth will be cleaned with rotating rubber cup and polishing paste. Thereafter, the subjects will be randomized to substitute their tobacco-based snus with either ZYN Dry or ZYN Moist for 4 weeks. The subjects will be provided with the IPs and will be instructed to use the products ad libitum, following their regular pattern of use, and to electronically register the number of ZYN pouches used per day, the time point, and the estimated usage time for four week (to Visit 5, Day 28).

On the third treatment visit (Visit 4, Day 14), a routine dental examination (including assessment of the amount of plaque and oral mucosal lesions), sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva for assessment of BoE will be performed. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and followed for 60 min. If needed, the subjects will be provided with more IPs (the subjects can also change flavor if they want).

On the fourth treatment visit (Visit 5, Day 28), a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva and urine for assessment of BoE will be performed. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and followed for 60 min. A biopsy of the oral mucosa will also be collected.

AEs will be collected by subject interview from the start of substitution of tobacco-based snus with ZYN nicotine pouches (Visit 3) until the last treatment visit (Visit 5).

**Investigational products (IPs) and dosage**

| IP | Nicotine content |
|---|---|
| ZYN Dry Smooth | 6 mg nicotine/pouch |
| ZYN Dry Cool Mint | 6 mg nicotine/pouch |
| ZYN Dry Citrus | 6 mg nicotine/pouch |
| ZYN Moist Smooth | 9 mg nicotine/pouch |
| ZYN Moist Cool Mint | 9 mg nicotine/pouch |
| ZYN Moist Citrus | 9 mg nicotine/pouch |

| |
|---|
| **Duration of treatment**<br>The participating subjects will be randomized to substitution of their tobacco-based snus with either ZYN Dry or ZYN Moist (these products will be chosen from a selection of one unflavored and two flavored products) for 4 weeks. The products will be used ad libitum. |
| **Duration of each subject's involvement in the study**<br>Each subject will participate in the study for 8 weeks, not including the preceding screening period. |
| **Assessment of dental plaque acidogenicity**<br>Plaque acidogenicity will be assessed by measuring pH in the plaque on two buccal surfaces in the upper jaw, one approximal surface in the upper jaw, and one approximal surface in the lower jaw before and up to 60 min after a 1 min mouth rinse with 10 mL of a 10% sucrose solution. |
| **Assessment of cariogenic microorganism in dental plaque**<br>Pooled plaque samples will be collected with a sterile toothpick from buccal areas and plated on different agar plates for analyses of the number of microorganisms related to caries and periodontal diseases. The number of colony-forming units will be counted. Also, for analyses of a broader range of cariogenic microorganisms, gene expression levels will be assessed. |
| **Amount of plaque assessment**<br>The amount of plaque will be measured on all surfaces of the Ramfjord teeth (16, 21, 24, 36, 41, and 46). For each tooth, six sites will be scored 0-3. |
| **Assessment of oral mucosal lesions**<br>Gingival hyperplasia will be assessed and graded (1-4) and clinical photos will be taken for comparisons at 4 time points (Day -28, 0, 14, and 28). After local anesthesia, a punch biopsy (4 mm in diameter) will be taken at two time points (Day 0 and Day 28) from the oral mucosa where the pouch previously was placed. The biopsy will be fixed in 10% formaldehyde, processed, mounted on glass slides, stained (Hematoxylin and Eosin, and other immunostainings when required), and subjected to histopathological evaluation and diagnosis by a specialist. |
| **Assessment of tooth discoloration**<br>Grading of tooth discoloration of the maxillary and mandibular anterior teeth will be performed on Day -28, Day 0 (before and after cleaning the teeth with rotating rubber cup and polishing paste), and on Day 28. Grading of discoloration will be assessed by colorimetry using a colorimeter (Minolta Chroma Meter CR-321). A visual shade evaluation will also be performed using a shade guide (VITA Zahnfabrik, Germany). |
| **Safety assessment**<br>AEs will be collected by subject interview from the start of IP administration (Visit 3) until the last treatment visit (Visit 5). |
| **Assessment of salivary secretion rate and buffer capacity**<br>The salivary secretion rate and buffer capacity will be assessed in unstimulated and stimulated saliva. Unstimulated salivary secretion rate will be assessed by collection of passively dropping saliva for 10 min. Stimulated salivary secretion rate will be assessed by collection of saliva (spit) for 5 min during which the subjects will chew on a piece of paraffin. pH will be assessed in unstimulated saliva by a pH meter and the buffer capacity will be assessed in the stimulated saliva using a commercially available kit (CRT Buffer). |
| **Assessments of BoE** |

| |
|---|
| BoE (*e.g.*, nicotine, cotinine, and tobacco-specific nitrosamines) will be assessed in saliva and urine collected on Visit 3 (Day 0), Visit 4 (Day 14, saliva only), and Visit 5 (Day 28). |
| **Statistical methods**<br><br>The primary endpoint is the difference between ZYN Dry and ZYN Moist in terms of changes in dental plaque acidogenicity, assessed by measuring pH in plaques, after substitution of tobacco-based snus for 4 weeks. No formal sample size calculation has been performed as available data for the two study product types are lacking. Based on previous experiences using this method, 20 subjects are considered to generate sufficient data for the purpose of this study. Hence, with an estimated dropout rate of 15% a total of 46 subjects will be randomized to achieve 40 fully evaluable subjects.<br><br>Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value, Q1-Q3 (interquartile range [IQR]).<br><br>Categorical data will be presented as counts and percentages. When applicable, summary data will be presented for the two arms (ZYN Dry and ZYN Moist), and by assessment time. Individual subject data will be listed by subject number, treatment arm, and, where applicable, by assessment time.<br><br>All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).<br><br>Baseline will be defined as the last data collected before subjects are randomized into substitution of tobacco-based snus with either ZYN Dry or ZYN Moist.<br><br>No adjustment for multiple comparisons will be made.<br><br>No imputation of missing data will be performed. |
| **Study reporting**<br><br>After completion of the study, an International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E3 compliant CSR will be prepared. |

## 2 TABLE OF CONTENTS

**List of Tables**

# 3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation | Explanation |
|---|---|
| ADL | Activities of daily living |
| AE | Adverse event (or adverse experience) |
| ATC | Anatomical therapeutic chemical |
| BoE | Biomarkers of exposure |
| CSP | Clinical study protocol |
| CSR | Clinical study report |
| CTC | Clinical Trial Consultants AB |
| CTCAE | Common terminology criteria for adverse events |
| DMP | Data management plan |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| EEA | European Economic Area |
| EMA | European Medicines Agency |
| FAS | Full analysis set |
| GCP | Good clinical practice |
| GDPR | General data protection regulation |
| ICF | Informed consent form |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IEC | Independent ethics committee |
| IME | Important medical event |
| IP | Investigational product |
| ISF | Investigator site file |
| IQR | Interquartile range |
| IUD | Intra-uterine device |
| IUS | Intra-uterine system |
| MedDRA | Medical dictionary for regulatory activities |
| MPA | Medical Products Agency |
| NP | Nicotine pouch |
| PI | Principal Investigator |
| PII | Personally Identifiable Information |
| PPE | Personal protective equipment |
| PT | Preferred term |

| | |
|---|---|
| PV | Pharmacovigilance |
| QA | Quality assurance |
| QC | Quality control |
| RBM | Risk-based monitoring |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SDV | Source data verification |
| SIL | Subject identification list |
| SOC | System organ class |
| SOP | Standard operating procedures |
| TMF | Trial master file |

# 4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

## 4.1 Medical emergencies contacts

The Principal Investigator (PI) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.6.9.

In the case of a medical emergency, the Investigator may contact the medically responsible person at Swedish Match (Table 4.1-1).

***Table 4.1-1 Medical emergencies contact***

| Name | Function in the study | Contact information |
|---|---|---|
| [redacted] | [redacted] | [redacted] |

# 5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

**Sponsor**

Swedish Match North Europe
Maria Skolgata 83
SE-118 53 Stockholm
Sweden

**Clinical conduct**

Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg
Box 450
SE-405 30 Gothenburg
Sweden

**Study management**

Clinical Trial Consultants AB (CTC)
Dag Hammarskjölds väg 10B
SE-752 37 Uppsala
Sweden

[redacted]

[redacted]

Signatures are provided in Section 19.

# 6 INTRODUCTION

## 6.1 Background

Snus is a tobacco-based product which has been used for long in Sweden. No increased caries risk is associated with snus use and no difference in terms of filled teeth has been found when comparing matched Swedish adolescent snus users and non-users (1). Instead, it has been reported that tobacco-based snus products can reduce biofilm acidogenicity and thus may protect against dental caries (2). This contrasts with American snuff and other smokeless products, which have been found to increase the risk of dental caries (3, 4). Gingival hyperplasia, known as snus-induced lesions, are commonly found among tobacco-based snus users (5). Tobacco-products (*e.g.*, cigarettes and some smokeless tobacco products) have also been reported to affect other periodontal tissues such as cementum, alveolar bone, and the periodontal ligament and to be associated with periodontal disease (6).

Today, there are several different types of smokeless nicotine-containing products, with the same use topography and systemic nicotine exposure as snus, found on the market. These products can be divided into tobacco- and non-tobacco-leaf containing products. During the last few years, the market for non-tobacco-leaf containing products, such as nicotine pouches (NPs), have grown tremendously and NP products are now sold under several brand names such as ZYN, VOLT, LYFT, VELO, and ON!.

ZYN is available in two types of product categories, ZYN Dry and ZYN Moist, and comes in various flavors and nicotine strengths. The nicotine content in ZYN (1.5-13 mg/unit) is comparable to that in snus and many other oral tobacco products that are currently common on the market in Scandinavia and the United States, which have nicotine contents ranging from 3 mg/unit to more than 20 mg/unit.

When comparing the nicotine content of different nicotine-delivery products, it is important to consider that the nicotine extraction and uptake varies considerably depending on product type (tobacco *vs.* a non-tobacco-based matrix) and product formulation (pouch geometry, solubility, water content, particle size, pH, *etc*.). In addition, there is a substantial inter-individual variation in uptake for products used orally, which is probably related to constitutional differences in saliva production, for example, and results in a wide variation in nicotine extraction. Previous studies (7) have found that snus (General PSWL) and ZYN Dry release approximately 32-33% and 50-60% of the nicotine during 60 min, respectively, although with large interindividual variation. Other studies (8-10) have found that ZYN Moist (also called ZYN ULTRA), which has a larger pouch dimension and higher moisture, salt, and nicotine content than ZYN Dry, releases approximately 32-42% of the nicotine during 60 min.

A previous clinical oral health study (11), in which healthy, current daily snus users were recommended to replace as much as possible of their snus with ZYN Dry during the 6-week study period, found that use of ZYN Dry is less harmful to the soft tissue and causes less snus-induced lesions. However, limited information is still present regarding ZYN´s effect on the oral environment, specifically variables related to dental caries and periodontal disease.

## 6.2 Study rationale

The overarching aim of the study is to clinically evaluate the effects on dental plaque acidogenicity and other oral variables in current, daily users of tobacco-based snus who completely substitute their snus use with either ZYN Dry or ZYN Moist. The rationale for the study design is presented in Section 8.2.

## 6.3 Risk/benefit assessment

### 6.3.1 *Risk assessment*

It may be considered problematic to expose subjects to novel nicotine delivery products with properties that are not yet fully known. However, all subjects are required to be daily tobacco-based snus users for at least one year (with an average or above-average consumption). Thus, the participants are well acquainted with and used to the effects of nicotine, and there will be no risk for the development of any novel nicotine dependency among the participants.

The nicotine in the ZYN products is of pharmaceutical grade, same as the nicotine used in nicotine replacement therapy products (*e.g.*, gum, lozenges, mouth spray etc.). Aside from nicotine, all ingredients used in the ZYN products are approved for use in food.

The potential adverse effects of the study procedures are likely to be minor and/or clinically insignificant, based on experience from clinical trials on similar products (12-15). The evidence on the health effects of nicotine exposure is based on existing tobacco products. In addition, any potential subject who intends to change their nicotine consumption habit or stop using nicotine products will not be offered the opportunity to participate in the study. Consequently, the present study is not perceived to confer any societal burden in terms of increased use of nicotine products.

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

The subjects will not be exposed to nicotine levels from the IPs that are higher than those they are usually exposed to during their daily consumption of tobacco-based snus. Therefore, acute risks are anticipated to be low. So far, no adverse events (AEs) have been reported in previous clinical studies with similar products, apart from effects likely to be related to nicotine exposure (such as salivation, nausea, and dyspepsia).

The assessments of dental plaque acidogenicity require participants to refrain from approximal tooth cleaning for 48 h and toothbrushing for 24 h prior to the examination, and the procedure involves a 1 min exposure to a 10% sucrose solution that will lower the plaque pH. This could theoretically have adverse effects on the subjects' dental health, but previous studies have shown that refraining from toothbrushing for such a short period of time does not adversely affect dental health.

Individuals with a saliva production below-average will also be excluded as AEs related to xerostomia could adversely influence outcomes and thus bias the study; however, it is expected that few, if any, of the subjects have problems related to xerostomia as the subjects will be current, daily snus users since more than one year.

The punch biopsy (4 mm in diameter) will be taken after application of local anesthesia according to the procedure for routine clinical biopsy sampling. This will not be associated with any major discomfort or significant AEs. Also, the procedures used to assess oral health, including measurements of dental plaque acidogenicity, are standard procedures used at odontological research facilities and will not be associated with any major discomfort or significant AEs. It is unlikely that these procedures will adversely affect the subject's long-term oral health because of the limited duration of these procedures. In fact, theoretically, participation in the study may help to improve the subjects' long-term oral health through an increased awareness of the significance of dental hygiene.

The PI at the research clinic will ascertain adequate facilities and procedures are available to handle any emergency situations that may occur during the study.

The potential AEs of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective, counterbalanced by reduced harm and risk of tobacco-related diseases of the novel NPs. As the market for this type of products is growing worldwide it is important to conduct formal clinical studies assessing effects on oral health, specifically variables related to dental caries and periodontal disease.

#### 6.3.2 *Benefit assessment*

In analogy with a regular phase I study in healthy subjects, there is no direct benefit for the subjects to participate in the study, aside from an oral examination, which may provide them with information on their general state of oral health. Hence, the safety and wellbeing of the subjects are of outmost importance.

The development of new, nicotine-containing products takes place both in the pharmaceutical industry and in the tobacco industry. Parts of the tobacco industry today are moving towards reducing the presence of known harmful substances, other than nicotine, in the products that are being developed. NP products such as ZYN are an example of such a development, and the use, prevalence and variety of these products has increased globally in recent years. NPs constitute a substitute to both combusted or non-combusted tobacco/nicotine-containing inhalation products (*e.g.*, conventional cigarettes, heated tobacco vaporizers or electronic cigarettes) and to oral tobacco products (*e.g.*, tobacco-based snus and moist snuff).

As mentioned in Section 6.1, there is a lack of scientific data regarding the effect on the oral environment, specifically variables related to dental caries and periodontal disease for oral nicotine products in general. Thus, the results generated from this study should be of interest not only for the tobacco industry and consumers, but also for lawmakers and the relevant regulatory authorities.

#### 6.3.3 *Risk/benefit conclusions*

It is reasonable to assume that NP products will have less harmful effects on oral health than tobacco-based snus products. From that perspective, the aim of the present study is consistent with society's overall goal of reducing the harm caused by tobacco use.

Overall, the potential benefit of developing and thoroughly characterizing improved tobacco-leaf free nicotine preparations is considered to outweigh the minimal risks that the subjects are exposed to in the study.

### 6.4 Risk assessment regarding the COVID-19 pandemic

Current recommendations from the authorities will be considered on a day-to-day basis and a continuous risk evaluation will be made to assess how the COVID-19 pandemic is affecting the study conduct and the safety of the study subjects. This study includes a healthy population. Hence, study participation should not contribute to increased risks on behalf of the safety of the study subjects or the quality of the data collected.

The recommendations from the European Medicines Agency (EMA) (16, 17) as well as the Swedish Medical Products Agency (MPA) (18) regarding the conduct and management of clinical trials during the COVID-19 pandemic will be taken into consideration. Currently identified risks as well as the planned prevention and mitigation actions are detailed in Table

6.4-1 and in a risk log as part of the Sponsor's trial master file (TMF). These may be updated in line with prevailing recommendations, as applicable.

***Table 6.4-1 COVID-19 related risks and mitigating actions***

| Risks identified | Actions |
|---|---|
| Subjects will be exposed to the SARS-CoV-2 virus, which may lead to subsequent development of COVID-19. | According to the recommendations by the Public Health Agency of Sweden (Folkhälsomyndigheten), subjects are not allowed to visit the clinic if they have any symptoms (even if not COVID-19 related). |
| | Subjects are supplied with hand disinfectant to use during visits to the clinic. |
| Subjects will fall ill with COVID-19 between visits and not be able to show up for study assessments within the assigned time window. | AEs will be followed up by phone. |
| | Subjects with suspected COVID-19 symptoms will be directed to seek COVID-19 testing. |
| | Subjects who are tested positive for COVID-19 between visits will be referred to standard hospital care if needed. |
| New recommended actions by health authorities that may halt the study, such as society lock-down, if the pandemic escalates. | This risk does not affect subject safety. |

# 7 STUDY OBJECTIVES AND ENDPOINTS

## 7.1 Primary objective

The primary objective of the study is to compare the impact on dental plaque acidogenicity after 4 weeks of substitution of tobacco-based snus with either ZYN Dry or ZYN Moist.

### 7.1.1 *Primary endpoint*

The difference between ZYN Dry and ZYN Moist in terms of changes in dental plaque acidogenicity, assessed by measuring pH in plaques, after substitution of tobacco-based snus for 4 weeks.

## 7.2 Secondary objectives

The secondary objectives of the study are:

1. To compare the effects on dental plaque acidogenicity following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. To compare the number of cariogenic microorganisms in dental plaque following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
3. To compare the amount of plaque following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
4. To evaluate the degree of oral mucosal lesions following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
5. To compare tooth discoloration following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
6. To evaluate the safety and tolerability of ZYN Dry and ZYN Moist in current, daily tobacco-based snus users.

### 7.2.1 *Secondary endpoints*

The secondary endpoints are:

1. The difference in dental plaque acidogenicity, assessed by measuring pH in plaques, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. The difference in the counted number of cariogenic microorganisms in dental plaque following tobacco-based snus substitution with either ZYN Dry or ZYN for 4 weeks.
3. The difference in the amount of plaque, counted and scored 0-3, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
4. The difference in the degree of oral mucosal lesions, by presence, visual grading, and histopathological evaluation of biopsies, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
5. The difference in tooth discoloration, by means of colorimetry using a colorimeter and by visual shade grading using a shade guide, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.

6. Frequency, intensity, and seriousness of AEs.

## 7.3 Exploratory objectives

1. To compare BoE in saliva following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. To compare BoE in urine following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.

The results of the exploratory objectives may not be reported in the clinical study report (CSR).

### 7.3.1 *Exploratory endpoints*

1. The difference in analyzed BoE in saliva following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.
2. The difference in analyzed BoE in urine following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks.

# 8 STUDY DESIGN

## 8.1 Overall study design and schedule of events

This is an open-label, two-armed, randomized, longitudinal study, designed to assess the effects on dental plaque acidogenicity and other oral variables in current, daily users of tobacco-based snus who completely substitute their snus use with either ZYN Dry or ZYN Moist nicotine pouches (NPs). The IPs are unflavored (Smooth) or flavored (Cool Mint and Citrus) NPs and of different nicotine strengths (ZYN Dry 6 mg and ZYN Moist 9 mg).

The study will include 46 randomized subjects with the aim to achieve 40 fully evaluable subjects. The subjects are healthy male or female tobacco-based snus users aged ≥21-≤55 years who have used tobacco-based snus for ≥1 year, with a minimum weekly consumption of 3 or more cans. An effort will be made to randomize at least 5 female subjects per treatment arm in the study (each arm consisting of 23 subjects in total).

The subjects report to the clinic for a screening visit (Visit 1) followed by 4 treatment visits (Visit 2-5) on separate days. The screening (Visit 1) will take place within 5 weeks prior to Visit 2 and will include an eligibility check, including evaluations of tobacco/nicotine use, a brief oral examination, urine pregnancy testing (women of childbearing potential only), and collection of medical history. Also, the salivary secretion rate and buffer capacity will be assessed (see Table 8.1-1 for details). At the end of the screening, the subjects will be asked about flavor preferences on IPs.

Before each treatment visits (Visit 2-5; Day -28, Day 0, Day 14, and Day 28, respectively), scheduled at the same time of the day for each subject, the subjects will refrain from approximal tooth cleaning for 48 h and toothbrushing for 24 h prior to the visits. Also, the subjects will be instructed not to eat, drink, chew gum, or any other mouth related procedures 1 h before the visits and during the visits.

On the first treatment visit (Visit 2, Day -28), the subjects will report to the clinic for a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), and sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva for assessment of BoE. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and assessed at 0, 2, 5, 10, 15, 30, 45, and 60 min. Oral hygiene and dietary habits will also be assessed by a questionnaire. Moreover, the subjects will be provided with a standard toothpaste containing fluoride (sodium fluoride-containing toothpaste; 1450 ppm F) that must be used by the subjects throughout the whole study. The subjects will use their regular toothbrush, which may be manual or electric, during the whole study. Finally, the teeth will be cleaned with rotating rubber cup and polishing paste. The subjects will electronically register the number of tobacco-based snus pouches used per day, the timepoints, and the estimated usage time for four weeks (to Visit 3, Day 0).

On the second treatment visit (Visit 3, Day 0), a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), and sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva and urine for assessment of BoE will be performed. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and assessed at 0, 2, 5, 10, 15, 30, 45, and 60 min. A biopsy of the oral mucosa will be collected. Finally, the teeth will be cleaned with rotating rubber cup and polishing paste. Thereafter, the subjects will be randomized to substitute their tobacco-based snus with either ZYN Dry or ZYN Moist for 4 weeks. The subjects will be provided with the

IPs and will be instructed to use the products ad libitum, following their regular pattern of use, and to electronically register the number of ZYN pouches used per day, the time point, and the estimated usage time for four week (to Visit 5, Day 28).

On the third treatment visit (Visit 4, Day 14), a routine dental examination (including assessment of the amount of plaque and oral mucosal lesions), sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva for assessment of BoE. Data regarding dental plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and assessed at 0, 2, 5, 10, 15, 30, 45, and 60 min.

On the fourth treatment visit (Visit 5, Day 28), a routine dental examination (including assessment of the amount of plaque, oral mucosal lesions, and tooth discoloration), sampling of specimen for counting the number of mutans streptococci and lactobacilli as well as collection of saliva and urine for assessment of BoE will be performed. Data regarding plaque acidogenicity will be collected before and after a 1 min mouth rinse with 10 mL of a 10% sucrose solution and assessed at 0, 2, 5, 10, 15, 30, 45, and 60 min. A biopsy of the oral mucosa will also be collected.

AEs will be collected by subject interview from the start of substitution of tobacco-based snus with ZYN (Visit 3) until the last treatment visit (Visit 5).

The total duration for each subject in the study will be 8 weeks, not including the preceding screening period.

See Table 8.1-1 for schedule of events applicable for each treatment visit.

Study assessments are described in Section 11.

***Table 8.1-1 Schedule of events***

| Events | Visit 1 Screening | Visit 2 First treatment visit (Day -28) | Visit 3 Second treatment visit (Day 0) | Visit 4 Third treatment visit (Day 14) | Visit 5 Fourth treatment visit (Day 28) |
|---|---|---|---|---|---|
| Informed consent | X | | | | |
| Demographics | X | | | | |
| Medical/surgical history | X | | | | |
| History of nicotine use | X | | | | |
| Inclusion/exclusion criteria | X | X[1] | | | |
| Brief oral examination | X | | | | |
| Salivary secretion rate and buffer capacity | X | | | | |
| Pregnancy test[2] | X | X[3] | X[3] | X[3] | X[3] |
| Baseline symptoms | X | X | | | |
| Question about flavor preferences | X | | | | |
| Prior and concomitant medications | X | | | | |
| Oral examination | | X | X | X | X |
| Plaque acidogenicity[4] | | X | X | X | X |
| Cariogenic microorganisms | | X | X | X | X |
| Amount of plaque | | X | X | X | X |
| Oral mucosal lesions | | X | X | X | X |
| Discoloration | | X | X[5] | | X |
| Collection of saliva | | X | X | X | X |
| Collection of urine | | | X | | X |
| Oral hygiene and dietary habits questionnaire | | X | | | |
| Use of standard toothpaste | | X | X | X | X |
| Cleaning with rotating rubber cup and polishing paste | | X | X | | |
| Randomization | | | X | | |
| Handout of IPs | | | X | X[6] | |
| Monitoring of consumption[7] | | X | | | |
| Biopsy of the oral mucosa | | | X | | X |
| AEs | | | X | X | X |

[1] Confirmation of eligibility criteria.
[2] Only subjects of childbearing potential.
[3] Only at the discretion of the Investigator. Only at the discretion of the PI on Visits 2-5.
[4] Assessed at 0, 2, 5, 10, 15, 30, 45, and 60 min after a 1 min mouth rinse with a 10% sucrose solution.
[5] Assessed before and after cleaning the teeth with rotating rubber cup and polishing paste.
[6] Only if necessary and/or if the subjects want to change flavor of the IP.
[7] Monitoring of the number of pouches (tobacco-based snus respectively IP) used per day, time points, and estimated usage time.

## 8.2 Rationale for study design

This is an open-label, two-armed, randomized, longitudinal study, designed to assess the effects on dental plaque acidogenicity and other oral variables in current, daily tobacco-based snus users who completely substitute their snus with either ZYN Dry or ZYN Moist NPs.

A parallel study design was chosen for comparison of two types of ZYN product categories, ZYN Dry and ZYN Moist.

Randomization will be used to minimize bias in the assignment of subjects to one of the two treatment arms and to increase the likelihood that known and unknown subject attributes (*e.g.*, demographic and baseline characteristics) will be evenly balanced.

# 9 STUDY POPULATION

Prospective approvals of protocol deviations from eligibility criteria, also known as protocol waivers or exemptions, are not permitted.

## 9.1 Recruitment

The subjects will be recruited from CTC's database of healthy volunteers, as well as from strategic marketing campaigns. Advertisements in social media and other media (newspapers, internet, radio, local distribution of flyers *etc.*) will be used to reach the target audience. The advertising texts approved by the independent ethics committee (IEC) will be used to create all the materials (digital, radio and/or print) for recruitment.

## 9.2 Screening and enrolment log

The PI 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 that were screened but not included. The reason for withdrawal should be stated for all subjects that were included but did not complete the study.

A screening number will be allocated to each subject in connection to the informed consent process at the screening visit (Visit 1). The screening number is generated automatically in the electronic case report form (eCRF). The screening number will allow identification of subjects irrespective of their eligibility for the study.

Subjects included and randomized will be assigned a randomization number (101, 102 *etc*.).

## 9.3 Number of subjects

Forty-six (46) subjects will be included in the study. Approximately 60 subjects will be screened with the aim of achieving 46 randomized subjects and 40 fully evaluable subjects. A fully evaluable subject is defined as a subject who has used the IPs for 4 weeks and who has completed all study visits. An effort will be made to randomize at least 5 female subjects to each treatment arm (each consisting of 23 subjects in total), however a minimum of 2 randomized female subjects in each treatment arm will be considered acceptable.

For replacements of subjects who discontinue from the study, see Section 9.8.3.

## 9.4 Inclusion criteria

To be included in the study, subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the study.
2. Subjects who have used tobacco-based snus for ≥1 year, with a minimum weekly consumption of 3 or more cans, and ≥90% of the total nicotine consumption should have been tobacco-based snus.
3. Non-smoker (have smoked maximum 5 packages in total ever and have not smoked at all during the last year).
4. Healthy male or female subject aged ≥21 to ≤55 years.

5. Normal stimulated salivary secretion rate (>0.7 mL/min).
6. At least 24 own teeth remaining and overall good oral health, as judged by the Investigator.
7. Female subjects of child-bearing potential must be willing to use a sufficient contraceptive method for the duration of the study, this includes mechanical barrier (*e.g.*, a male condom or a female diaphragm), combined [estrogen and progestogen containing] hormonal contraception associated with inhibition of ovulation [oral, intravaginal, transdermal], progestogen-only hormonal anticonception associated with inhibition of ovulation [oral, injectable, implantable], intra uterine device (IUD) or intra uterine system (IUS). Sexual abstinence is allowed when this is the preferred and usual lifestyle of the subject.

### 9.5 Exclusion criteria

Subjects must be excluded from the study if any of the following exclusion criteria are fulfilled:

1. A history of diagnosed hypertension or any cardiovascular disease, or ongoing manifestations of hypertension or any cardiovascular disease as judged by the Investigator.
2. Any surgical or medical condition, including abnormal salivation (also pharmaceutically induced), or history thereof, which, in the judgment of the Investigator, might interfere with the absorption, distribution, metabolism or excretion of the IP or may either put the subject at risk because of participation in the study, influence the results, or the subject's ability to participate in the study.
3. Subjects who are pregnant, breastfeeding, or intend to become pregnant during the course of the study.
4. A history of diagnosed severe allergy/hypersensitivity or ongoing manifestations of severe allergy/hypersensitivity to aroma compounds (including fragrances and/or flavorings), as judged by the Investigator.
5. Subjects with severe oral conditions such as open caries lesions, severe periodontal health, lesions in the soft tissues (apart from gingival hyperplasia related to the use of snus), or extensive prosthetic work (*e.g.*, several implants, partial denture, dental veneers).
6. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse, as judged by the Investigator.
7. Antibiotic use ≤4 weeks prior to the screening period.
8. Subjects who intend to change their nicotine consumption habit, including the intention to stop using nicotine products, within the next 4 months of the screening visit, as judged by the Investigator.
9. Subjects undergoing other dental treatment during the study period.
10. The Investigator considers the subject unlikely to comply with study procedures, restrictions, and requirements.

## 9.6 Restrictions during the study

The subjects must be willing to comply with the following restrictions during the entire study duration, *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 5).

### 9.6.1 *General restrictions*

1. Subjects shall refrain from approximal tooth cleaning for 48 h and toothbrushing for 24 h prior to the treatment visits (Visit 2-Visit 5).
2. Subjects shall abstain from eating, drinking, chew gum, or any other mouth related procedures for 1 h prior to the treatment visits (Visit 2-5).
3. Subjects shall completely substitute their tobacco-based snus product for ZYN from the second treatment visit (Visit 3) and abstain from all other nicotine products until the end of the study (Visit 5).
4. Subjects shall abstain from any drugs of abuse during the study, *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 5).
5. Subjects shall abstain from alcohol for at least 12 h prior to each treatment visit (Visit 2-5).
6. Subjects shall use their regular toothbrush (electrical or manual) and the standard toothpaste containing fluoride (sodium fluoride-containing toothpaste; 1450 ppm F) from Visit 2 to Visit 5.
7. Subjects are not allowed to participate in any other clinical studies or use antibiotics during the study period, *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 5).

### 9.6.2 *Prior and concomitant therapy*

Subjects undergoing other dental treatment during the study period will be excluded from the study. There will be no other restrictions concerning concomitant medications or therapies, as long as the subject is on a stable course of medication for the duration of the study. Prescribed medications taken *pro re nata* may be a reason for exclusion as judged by the Investigator if they affect the subject's general condition or salivation.

## 9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently visiting the clinic on the first treatment visit. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

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

- Practical reasons.
- Non-significant medical conditions (*e.g.*, influenza, nasopharyngitis).

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

## 9.8 Subject withdrawal

### 9.8.1 *General withdrawal criteria*

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

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

Reasons for discontinuation include:

1. Severe non-compliance with CSP procedures, as judged by the Investigator and/or Sponsor.
2. Subject is lost to follow-up. A subject will be considered lost to follow-up if he/she fails to come for consecutive scheduled visits and if he/she is not possible to be contacted by site staff despite several attempts.
3. Significant AE posing a risk for the subject, as judged by the Investigator and/or Sponsor.
4. Pregnancy.

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

If a subject withdraws consent or prematurely discontinues participation in the study, they will always be asked about the reason(s) for discontinuation/early withdrawal and the presence of any AEs. Any ongoing AEs will be followed as described in Section 11.6.10.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF. If the primary reason in an AE, the AE must be specified.

### 9.8.3 *Subject replacement*

Subjects who are prematurely withdrawn from the study may be replaced at the Sponsor's discretion after consultation with the PI. Subjects prematurely withdrawn due to AE(s) assessed as at least possibly related to the IP will not be replaced.

## 9.9 Randomization

On Visit 3, the subjects will be randomized into two treatment arms (ZYN Dry or ZYN Moist). Within each treatment arm, the subjects can choose flavor and also change the selected flavor during the study. As this is an open-label study, the treatment arm to which each subject is allocated will be recorded in the eCRF. A computer-generated randomization list will be created using SAS Proc Plan, SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC). The randomization list will contain subject number and treatment arm.

The randomization list will be generated by CTC and a copy of the randomization list will be provided to the packing company and to the clinic. The original randomization list will be kept by the randomizer.

Because this is an open-label study, no blinding procedures will be followed.

# 10 TREATMENTS

The IPs are supplied by Swedish Match.

## 10.1 Identity of test products

The test products that will be used in the study are detailed in

Table 10.1-1.

***Table 10.1-1 Identity of IPs***

| IP | Nicotine content |
|---|---|
| ZYN Dry Smooth | 6 mg nicotine/pouch |
| ZYN Dry Cool Mint | 6 mg nicotine/pouch |
| ZYN Dry Citrus | 6 mg nicotine/pouch |
| ZYN Moist Smooth | 9 mg nicotine/pouch |
| ZYN Moist Cool Mint | 9 mg nicotine/pouch |
| ZYN Moist Citrus | 9 mg nicotine/pouch |

## 10.2 Manufacturing and packaging of the IPs

All IPs are manufactured and packaged by Swedish Match in compliance with the Swedish law on food production. Production sites and batch IDs for the IPs will be documented in the TMF. IPs will be shipped by Swedish Match directly to the research clinic (Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden).

## 10.3 Conditions for storage

All IPs will be stored at room temperature (20-25 °C) in an access-restricted storage area at the Department of Cariology.

## 10.4 Preparation and accountability

The IPs will be distributed according to the randomization list by the site personnel. The Investigator will maintain a storage and accountability log as well as a distributing log detailing the dates and quantities of study IPs provided to each subject, as well as any IPs returned to the Sponsor at the end of the study. Products deliberately and/or accidentally destroyed by the site or the subject must be accounted for.

## 10.5 Treatment administration

The subjects will exchange their tobacco-based snus with the randomized ZYN product and will be instructed to use the products ad libitum, following their regular pattern of use. The compliance will be checked by asking the subjects. The subjects will electronically register the number of pouches used per day, the time points, and the estimated usage time. There will

be a variation in flavors of the products offered. The pouches will be placed in the upper vestibulum.

### 10.6 Treatment compliance

Any unused study product will be collected and kept at site until returned to the Sponsor. The Monitor will perform a final IP accountability reconciliation at the end of the study to verify that all unused IP have been returned to the Sponsor and documented.

# 11 STUDY ASSESSMENTS

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

## 11.1 Recording of data

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

The different study assessments will be collected in order:

1. Dental plaque acidogenicity
2. Amount of plaque
3. Pooled dental plaque (cariogenic microorganisms)
4. Collection of saliva
5. Oral mucosa lesions
6. Tooth discoloration
7. Biopsy

The subjects will be scheduled for 4 treatment visits (Visit 2-5), Day -28, Day 0, Day 14, and Day 28. The allowed time deviation for these visits is ±2 days. The subjects will collect the morning urine and transfer it to the clinic on Visit 3 and Visit 5. For plaque acidogenicity, assessments will be performed before and after a mouth rinse with 10% sucrose for 1 min and pH will be measured in the plaques at the time points 0, 2, 5, 10, 15, 30, 45, and 60 min. Allowed deviations for these time points are ±1 min for the time points 0-15 min and ±2 min for the time points 30-60 min.

## 11.2 Demographics and other baseline characteristics

### 11.2.1 *Informed consent*

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

### 11.2.2 *Demographic information*

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

### 11.2.3 *Medical/surgical history*

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

### 11.2.4 *History of nicotine use*

The history of nicotine uses in terms of brands, average consumption per day during the last 30 days, and duration of use (years), and history of smoking (cigarettes and e-cigarettes) will be obtained by subject interview.

#### 11.2.5 *Eligibility criteria*

Eligibility criteria should be checked during screening and verified before randomization on Visit 3. The criteria are specified in Sections 9.4 and 9.5.

#### 11.2.6 *Oral examination*

A brief oral examination will be performed at screening (Visit 1) and include assessments of overall good oral health status, normal stimulated salivary secretion rate (>0.7 mL/min), and at least 24 own teeth remaining. A brief oral examination will also be performed at each treatment visit to ensure overall good oral health status.

#### 11.2.7 *Pregnancy test*

All female subjects of childbearing potential will do a urine pregnancy test at screening (Visit 1) as well as at the discretion of the Investigator during the treatment visits (Visit 2-5).

#### 11.2.8 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until the start of the randomization on the second treatment visit (*i.e.*, an event that occurs when the subjects are using their tobacco-based snus). Such events are not AEs and will be recorded as baseline symptoms in the Medical History Log in the eCRF.

#### 11.2.9 *Prior and concomitant medication*

Prior and concomitant medications taken within 2 weeks prior to screening will be obtained by subject interview for documentation of the subject's status regarding current medications.

Medications will be classified as prior if the stop date was before or on the day of the randomization, prior to start of study product use, and as concomitant if ongoing on the day of the second treatment visit (Visit 3), started or stopped after the assessments on that visit. To distinguish between prior and concomitant medications on the second treatment visit (Visit 3), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of concomitant medication from screening until the last treatment visit (Visit 5) will be documented appropriately in the subject's eCRF. Relevant information (*i.e.*, name of medication, dose, unit, frequency, start and stop dates, reason for use) must be recorded. All changes in medication will be noted in the eCRF.

### 11.3 Assessment related to the primary endpoint

#### 11.3.1 *Dental plaque acidogenicity*

An iridium microelectrode 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 a pH meter (Orion SA720 pH/ISE Meter) to which also a reference electrode will be connected. The reference electrode is placed into a solution of 3 M KCl to which the subject´s finger is placed in order to create a salt bridge. After calibration and baseline assessment (0 min), the subjects will rinse their mouth with 10 mL of a 10% sucrose solution for 1 min and pH will then be measured at eight time points up to 60 min (0, 2, 5, 10, 15, 30, 45, and 60 min). In case of lack of microelectrodes, the strip method will be used; a paper indicator strip

will be inserted in the area and kept in place for 10 sec after which the color change will be manually compared with a color scale.
The date and time of collection of each sample and the assessed pH values will be recorded in the eCRF.

## 11.4 Assessments related to secondary endpoints

### 11.4.1 *Dental plaque acidogenicity*

The difference in dental plaque acidogenicity, assessed by measuring pH in plaques, following tobacco-based snus substitution with either ZYN Dry or ZYN Moist for 4 weeks will be assessed as described above for the primary endpoint.

### 11.4.2 *Cariogenic microorganisms in dental plaque*

Pooled plaque samples will be collected with a sterile toothpick (19) from the buccal areas of respective quadrants. The samples, handled within 1 h after collection, will be dispersed on a Whirlimixer, diluted in potassium phosphate buffer, and plated in duplicate on different agar plates for analyses of the number of microorganisms related to caries and periodontal diseases. MSB agar will be used for mutans streptococci, MS agar for total streptococci, Rogosa SL agar for lactobacilli, and blood agar for total viable count. After incubation, the number of colony-forming units (CFU) on the agar plates will be counted. For analyses of a broader range of cariogenic microorganisms, gene expression levels will be assessed by quantitative PCR.
The date and time of collection of each sample and the assessed CFUs will be recorded in the eCRF.

### 11.4.3 *Amount of plaque*

The amount of plaque will be measured on all surfaces of the Ramfjord teeth (16, 21, 24, 36, 41, and 46) according to Ramfjord (20). For each tooth, six sites (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual) will be scored from 0-3 according to Silness and Löe (21).
The date and time of collection of each sample and the assessed scores will be recorded in the eCRF.

### 11.4.4 *Oral mucosal lesions*

Gingival hyperplasia will be assessed and graded 1-4 according to Axell *et al.* (22) and clinical photographs will be taken (Day -28, 0, 14, and 28). On Day 0 and Day 28, after application of local anesthesia, a punch biopsy (4 mm in diameter) will be taken according to the procedure for routine clinical biopsy sampling. The biopsy will be taken from the oral mucosa where the pouch previously was placed. The biopsy will be fixed in 10% formaldehyde, processed, and mounted on glass slides, and stained with Hematoxylin and Eosin and immunostaining techniques when required. The histopathological evaluation and diagnosis will be made by a specialist within the field of oral medicine.
The date and time of collection of each sample, scores, and results from the histopathological evaluation will be recorded in the eCRF. The photographs will be stored on a computer, and only the Investigator can access the electronic records.

#### 11.4.5 *Tooth discoloration*

Grading of tooth discoloration of the maxillary and mandibular anterior teeth will be performed on Day -28, Day 0, and Day 28. On Day 0, it will be performed before and after cleaning of the teeth with rotating rubber cup and polishing paste. Grading of discoloration will be assessed by colorimetry using a colorimeter (Minolta Chroma Meter CR-321). Visual shade evaluation will also be performed using a shade guide (VITA Zahnfabrik, Bad Sackingen, Germany).
The date and time of collection of each sample, assessed values by colorimetry and results from shade evaluation will be recorded in the eCRF.

### 11.5 Assessments related to exploratory endpoints

#### 11.5.1 *Biomarkers of exposure in saliva and urine*

BoE (*e.g.*, nicotine, cotinine, and tobacco-specific nitrosamines) will be assessed in saliva and urine. Saliva (spit) will be collected at Visit 2-5. The subjects will collect the morning urine and transfer it to the clinic on Visit 3 and Visit 5. Saliva and urine will be stored at -20°C until analysis.

### 11.6 Adverse events

The PI is responsible for ensuring that all medical staff involved in the study are familiar with the content of this section.

#### 11.6.1 *Definition of adverse event*

An adverse event (also known as adverse experience), abbreviated AE, is defined as any untoward medical occurrence in a subject administered a medicinal product (in this case an IP) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the IP.

#### 11.6.2 *Definition of serious adverse event*

An SAE is any AE which:

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

outcomes defined above).

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

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

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

### 11.6.3 *Time period and frequency for collecting adverse events*

All AEs (including SAEs) will be collected by subject interview from the start of substitution of the subject's traditional tobacco-based snus with either ZYN Dry or ZYN Moist on Visit 3 (Day 0) until the last treatment visit (Visit 5, Day 28).

Any AE with start date on the day of the substitution of tobacco-based snus with ZYN must be recorded with start time.

At Visit 5, information on new AEs or SAEs, if any, and stop dates for ongoing events must be recorded as applicable.

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

### 11.6.4 *Assessment of intensity*

The grading of the intensity of AEs will follow the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 (23). 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 intensity of an AE using the definitions in Table 11.6-1, and record it on the AE log in the eCRF.

***Table 11.6-1 Grading of adverse event intensity***

| Grade | Definition |
|---|---|
| **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)[1]. |
| **Grade 3** | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2]. |
| **Grade 4** | Life-threatening consequences: urgent intervention indicated. |
| **Grade 5** | Death related to AE. |

[1] Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, et c.
[2] Self-care ADL refers to bathing, dressing, and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

### 11.6.5 *Assessment of causal relationships*

The Investigator must assess the causal relationship between an AE and the IP using the definitions in Table 11.6-2 and record it the AE log of the eCRF.

***Table 11.6-2 Assessment of adverse event causal relationship***

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

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

### 11.6.6 *Assessment of outcome*

The Investigator must assess the outcome of an AE using the definitions outlined in Table 11.6-3 and record it on the AE log of the eCRF.

***Table 11.6-3 Outcomes of adverse events***

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

### 11.6.7 *Collecting adverse events*

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

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

### 11.6.8 *Recording adverse events*

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

If the AE is serious (*i.e.*, an SAE), this must be indicated in the eCRF.

AEs must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

### 11.6.9 *Reporting of serious adverse events*

SAE reporting should be performed by the Investigator within 24 h of awareness via the eCRF. All available information regarding the SAE should be entered in the AE log for the specific subject. By saving the event as "serious" in the eCRF, and once the Investigator has signed-off of the event, an e-mail alert is automatically sent to predefined recipients to highlight that an SAE has been registered. The same information is automatically sent to [redacted]

The SAE report is reviewed by a designated person at CTC's pharmacovigilance (PV) department to ensure that the report is valid and correct. For fatal or life-threatening SAEs where important or relevant information is missing, immediate follow-up is undertaken and queries to the site are raised. Investigators or other site personnel should inform CTC's PV department of any follow-up information on a previously reported SAE immediately but no later than the end of the next business day of when they become aware of it.

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

If any additional documentation is required (*e.g.*, autopsy report), CTC's PV department will request this information from the study site.

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

[redacted]

A copy of the SAE form must also be e-mailed to CTC [redacted]

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

### 11.6.10 *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 until the end of the study, whichever comes first. At each subject's end of study visit (*i.e.*, the last treatment visit, Visit 5), information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs

assessed as stable by the Investigator at the end of the study 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.

#### 11.6.11 *Procedures in case of pregnancy*

In case of pregnancy or suspicion of pregnancy of any subject, 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 the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even after the subject was discontinued from the study.

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

#### 11.6.12 *Treatment of overdose*

Over-dosing is not applicable as the subjects will be instructed to follow their regular pattern of use. The consumption will be monitored via an app, and the subjects will report the number of pouches used per day, the time point for usage and length of each episode. In cases of accidental overdose, standard supportive measures should be adopted as required.

### 11.7 Appropriateness of measurements

All methods used for safety assessments are commonly used in standard medical care and in Phase I clinical studies.

# 12 PROCEDURES FOR BIOLOGICAL SAMPLES

## 12.1 Sample collections

The sample collection procedures for dental plaque acidogenicity, cariogenic microorganisms in plaque, number of plaques, gingival biopsies, saliva, and urine are described in Section 11.3.1.

## 12.2 Handling, storage, and destruction of laboratory samples

Gingival biopsies, saliva, and urine will be registered in a biobank, Biobank West (registration number 890 at the Health and Social Care Inspectorate, IVO). The samples will be stored at -20°C until analyzed and disposed of after the CSR has been finalized.

Any remains from other samples will be disposed of after analyses.

## 12.3 Chain of custody of biological samples

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

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

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

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

## 12.4 Withdrawal of informed consent for donated biological samples

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

The PI will ensure that:

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

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

# 13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

## 13.1 Quality management: critical process, system, and data identification

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

Identified risks will be categorized separately from the CSP.

## 13.2 Quality assurance and quality control

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

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

The Sponsor is responsible for implementing a risk-based validated EDC system and maintaining SOPs for the whole life cycle of the system.

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

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

# 14 ETHICAL AND REGULATORY REQUIREMENTS

## 14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (24) and are consistent with the ICH E6(R2) guideline for GCP (25), the EU Clinical Trials Directive (26), and applicable local regulatory requirements.

## 14.2 Ethics and regulatory review

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

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

The Sponsor will provide the IEC and PI with safety updates/reports according to local requirements.

## 14.3 Subject information and consent

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

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

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

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

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

## 14.4 Subject data protection

The ICF includes information that data will be recorded, collected, and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union general data protection regulation (GDPR), Regulation (EU) 2016/679 (27), the data will not identify any persons taking part in the study.

The potential study subject should be informed that by signing the ICF he/she approves that authorized representative from the Sponsor and CTC and the concerned IEC have direct access to his/her medical records for verification of clinical study procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete in accordance with Regulation (EU) 2016/679 (27) and the request will be raised to the PI.

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

Personal data that is collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudo-anonymized, *i.e.*, personally identifiable information (PII) will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the study.

For this study, the Sponsor Swedish Match is the data controller of all data processed during the study (*e.g.*, TMF and study reports) and CTC is the data processor. Any subcontractors used in the study are also data processors.

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

### 14.5 Changes to the approved clinical study protocol

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

### 14.6 Audits and inspections

Authorized representatives of the Sponsor may perform audits at the research clinic, including source data verification (SDV). The purpose of an audit is to examine all study-related activities and documents systematically and independently 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

Subjects will be covered under Swedish Match´s liability insurance policy through IF insurances. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. University of Gothenburg has a company insurance covering services performed at the laboratory. CTC has a company insurance covering services (*e.g.*, data management) 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 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 key staff to whom study-specific duties are delegated.

## 15.2 Clinical monitoring

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

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor, and provided separately, the responsible Monitor will periodically visit the study site at times agreed upon by the Investigator and the Monitor. At the time of each monitoring visit, the role of the Monitor is (but not limited) to:

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

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

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

### 15.3 Source data documents

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

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

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

### 15.4 Study agreements

The PI must comply with all the terms, conditions, and obligations of the clinical study agreement for this study.

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

### 15.5 Study timetable and end of study

The study is expected to start in Q4 2022 and to be completed by Q3 2023.

A subject is considered to have completed the study if he/she has completed all treatment visits in the study (Visit 2-5). Each subject who completes the study will participate in the study for a period of 8 weeks, not including the preceding screening period.

The end of the study is defined as the last visit, *i.e.*, the last treatment visit (Visit 5), of the last subject participating in the study.

### 15.6 Termination of the study

The Sponsor reserves the right to terminate this study prematurely for any reasonable cause. Conditions that may warrant study termination include but are not limited to a decision by the Sponsor to suspend or discontinue development of the IP.

If the study is prematurely terminated or suspended for any reason, the Investigator should promptly inform the study subjects and should assure appropriate follow-up for the subjects.

## 15.7 Reporting and publication

### 15.7.1 *Clinical study report*

A CSR, in compliance with ICH-E3, describing the conduct of the study, any statistical analyses performed, and the results obtained, will be prepared by the Sponsor in collaboration with CTC. The CSR will be reviewed and approved by, as a minimum, the PI, the statistician, and the Sponsor.

### 15.7.2 *Confidentiality and ownership of study data*

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

### 15.7.3 *Publication*

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

## 15.8 Archiving

The PI 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 SIL (providing the sole link between named subject source records and anonymous eCRF data), the original signed ICFs and detailed records of disposition of IP.

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

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

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

The completed eCRF are the sole property of the Sponsor 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 the Sponsor.

# 16 DATA MANAGEMENT

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

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

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

## 16.1 The web-based eCRF

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

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

## 16.2 The entering of data into the eCRF

All entries, corrections, and alterations in the eCRF are to be made by the Investigator or designated site personnel. Neither the Monitor nor any other study team member besides site personnel may enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. If some assessments are not done, or if certain information is not available, not applicable, or unknown, the Investigator or assigned clinical staff should record such information in the eCRF.

The Investigator must verify that all data entries in the eCRFs are accurate and correct and will be required to electronically sign off the clinical data. This will be performed by means of

the Investigator's unique User ID and password; date and time stamps will be added automatically at the time of electronic signature.

### 16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there are no discrepancies for critical data as described in the RBM plan.

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

### 16.4 Audit trail

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

### 16.5 External data

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

### 16.6 Medical coding

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

### 16.7 Database lock

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

# 17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

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

## 17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value, Q1-Q3 (interquartile range [IQR]).

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by each group (ZYN Dry or ZYN Moist), and by assessment time. Individual subject data will be listed by subject number, treatment arm (ZYN Dry or ZYN Moist), and, where applicable, by assessment time.

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

Baseline will be defined as the last data collected before subjects are randomized into substitution of tobacco-based snus with either ZYN Dry or ZYN Moist.

No adjustment for multiple comparisons will be made.

No imputation of missing data will be performed.

## 17.2 Determination of sample size

The primary endpoint is the difference between ZYN Dry and ZYN Moist in terms of changes in dental plaque acidogenicity, assessed by measuring pH in plaques, after substitution of tobacco-based snus for 4 weeks. No formal sample size calculation has been performed as available data for the two study product types are lacking. Based on previous experiences using this method, 20 subjects are considered to generate sufficient data for the purpose of this study. Hence, with an estimated dropout rate of 15% a total of 46 subjects will be randomized to achieve 40 fully evaluable subjects.

## 17.3 Analysis data sets

### 17.3.1 *Full analysis set*

The Full Analysis Set (FAS) will consist of all subjects who have been randomized. This population will be used as the safety analysis set.

### 17.3.2 *Additional analysis datasets*

Additional datasets may be created as needed for exploratory analyses.

## 17.4 Description of study population

### 17.4.1 *Demographics and baseline characteristics*

Demographics, as well as the history of nicotine use, will be presented by descriptive statistics. All data will be listed by subject.

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

Medical/surgical history will be presented by system organ classes (SOC) and preferred term (PT). Prior/concomitant medications will be presented by ATC level 1, 3 and 5 through descriptive statistics and listings.

### 17.4.3 *Treatment compliance*

The number of subjects treated with either ZYN Dry or ZYN Moist will be presented through descriptive statistics and listings.

### 17.4.4 *Oral examination*

Any abnormal findings at screening, *i.e.,* judged as outside the normal ranges by the Investigator, will be categorized as “abnormal, not clinically significant” or “abnormal, clinically significant” and presented in listings.

## 17.5 Analysis of the primary endpoint

### 17.5.1 *Difference in dental plaque acidogenicity between ZYN Dry and ZYN Moist*

The primary endpoint is the difference between ZYN Dry and ZYN Moist in terms of changes in dental plaque acidogenicity, assessed by measuring pH in plaques, after substitution of tobacco-based snus for 4 weeks. Assessment of dental plaque acidogenicity will be described using AUC6.2 to the observed dental plaque acidogenicity and presented using summary statistics as described in Section **17.1**.

## 17.6 Analysis of secondary endpoints

### 17.6.1 *Dental plaque acidogenicity*

The difference in dental plaque acidogenicity will be calculated from the start of tobacco-based snus substitution with either ZYN Dry or ZYN Moist (Visit 3) until the end of the study (Visit 5). The assessment of dental plaque acidogenicity will be described using AUC6.2 to the observed dental plaque acidogenicity and presented using summary statistics

### 17.6.2 *Number of cariogenic microorganisms in dental plaque*

The difference in the number of cariogenic microorganisms in dental plaque will be evaluated, graded, and calculated from the start of tobacco-based snus substitution with either ZYN Dry or ZYN Moist (Visit 3) until the end of the study (Visit 5) and presented through descriptive statistics.

#### 17.6.3 *Amount of plaque*

The difference in the amount of plaque will be calculated and scored 0-3 from the start of tobacco-based snus substitution with either ZYN Dry or ZYN Moist (Visit 3) until the end of the study (Visit 5) and presented through descriptive statistics.

#### 17.6.4 *Degree of oral mucosal lesions*

The difference in the degree of oral mucosal lesions will be assessed from the grading (1-4), clinical photographs, and the evaluation and diagnosis of the oral mucosa biopsies. Data will be summarized and presented through descriptive statistics.

#### 17.6.5 *Tooth discoloration*

The difference in tooth discoloration will be assessed by colorimetry (using a colorimeter) and by visually shade grading (using a shade scale) and presented through descriptive statistics.

#### 17.6.6 *Analysis of adverse events*

An overview of all AEs, including SAEs, intensity, and deaths will be presented by SOC and PT. The incidences of AEs and SAEs will be summarized by SOC and PT by IP.

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

### 17.7 Analysis of exploratory endpoints

#### 17.7.1 *Biomarkers of exposure in saliva*

The difference in BoE in saliva (*e.g.*, nicotine, cotinine, nornicotine, and tobacco-specific nitrosamines) will be calculated from the start of tobacco-based snus substitution with either ZYN Dry or ZYN Moist (Visit 3) until the end of the study (Visit 5) and summarized and presented through descriptive statistics.

#### 17.7.2 *Biomarkers of exposure in urine*

The difference in BoE in urine (*e.g.*, nicotine, cotinine, nornicotine, and tobacco-specific nitrosamines) will be calculated from the start of tobacco-based snus substitution with either ZYN Dry or ZYN Moist (Visit 3) until the end of the study (Visit 5) and summarized and presented through descriptive statistics.

## 18 REFERENCES

1. Rolandsson M, Hellqvist L, Lindqvist L, Hugoson A. Effects of snuff on the oral health status of adolescent males: a comparative study. Oral Health Prev Dent. 2005;3(2):77-85.
2. Hellqvist L, Bostrom A, Lingstrom P, Hugoson A, Rolandsson M, Birkhed D. Effect of nicotine-free and nicotine-containing snus on plaque pH in vivo. Swed Dent J. 2012;36(4):187-94.
3. Wendt LK, Birkhed D. Dietary habits related to caries development and immigrant status in infants and toddlers living in Sweden. Acta Odontol Scand. 1995;53(6):339-44.
4. Lee PN. Summary of the epidemiological evidence relating snus to health. Regul Toxicol Pharmacol. 2011;59(2):197-214.
5. Andersson G, Axell T, Larsson A. Histologic changes associated with the use of loose and portion-bag packed Swedish moist snuff: a comparative study. J Oral Pathol Med. 1989;18(9):491-7.
6. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809-20.
7. Lunell E, Fagerstrom K, Hughes J, Pendrill R. Pharmacokinetic Comparison of a Novel Non-tobacco-Based Nicotine Pouch (ZYN) With Conventional, Tobacco-Based Swedish Snus and American Moist Snuff. Nicotine Tob Res. 2020;22(10):1757-63.
8. Swedish Match Europe Division. ISRCTN13527565: A study investigating the uptake to the blood circulation of nicotine from tobacco-free nicotine pouches compared to American moist snuff pouches. isrctn.com. Applied January 23, 2020. Last edited January 10, 2022. [Available from: https://www.isrctn.com/ISRCTN13527565.
9. Swedish Match Europe Division. ISRCTN30119403: A study investigating the extraction of nicotine and flavors from tobacco-free nicotine pouches. isrctn.com. Applied February 9, 2021. Last edited June 14, 2022. [Available from: https://www.isrctn.com/ISRCTN30119403.
10. Swedish Match Europe Division. ISRCTN66329631: A study investigating the uptake to the blood circulation and subjective effects of nicotine from tobacco-free nicotine pouches. isrctn.com. Applied February 8, 2021. Last edited September 1, 2021. [Available from: https://www.isrctn.com/ISRCTN66329631.
11. Swedish Match Europe Division. ISRCTN16087707: An open observational study investigating the effect on oral health when using tobacco free nicotine pods. isrctn.com. Applied November 22, 2017. Last edited December 8, 2019. [Available from: https://www.isrctn.com/ISRCTN16087707.
12. Swedish Match Europe Division. ISRCTN44913332: A study investigating the extraction of nicotine and flavors from tobacco free nicotine pods compared to tobacco based Swedish snus. isrctn.com. Applied November 14, 2017. Last edited February 15, 2022. [Available from: https://www.isrctn.com/ISRCTN44913332.
13. Swedish Match Europe Division. ISRCTN77807609: A study investigating the uptake to the blood circulation of nicotine from tobacco free nicotine pods compared to tobacco-based Swedish snus and American moist snuff. isrctn.com. Applied October 3, 2018. Last edited May 28, 2020. [Available from: https://www.isrctn.com/ISRCTN77807609.
14. Swedish Match Europe Division. ISRCTN14866695: A study investigating the uptake to the blood circulation and subjective effects of nicotine from tobacco free nicotine pods compared to tobacco based Swedish snus. isrctn.com. Applied November 15, 2017. Last edited May 28, 2020. [Available from: https://www.isrctn.com/ISRCTN14866695.

15. Lunell E, 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):573-8.
16. European Medicines Agency. Points to consider on implication of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials. Ema.Europa.eu. 26 June 2020. EMA/158330/2020 Rev. 1. [Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/points-consider-implications-coronavirus-disease-covid-19-methodological-aspects-ongoing-clinical_en-0.pdf.
17. European Medicines Agency. Guidance on the management of clinical trials during the COVID-19 (coronavirus) pandemic. Ec.Europa.eu. 28 April 2020. Version 4. [Available from: https://ec.europa.eu/health/system/files/2022-02/guidanceclinicaltrials_covid19_en_1.pdf.
18. Swedish Medical Products Agency. Kliniska prövningar under covid-19-pandemin. Lakemedelsverket.se. December 18, 2020. Last updated February 15, 2021. [Available from: https://www.lakemedelsverket.se/sv/coronavirus/tillfalliga-regler/kliniska-provningar.
19. Kristoffersson K, Bratthall D. Transient reduction of Streptococcus mutans interdentally by chlorhexidine gel. Scand J Dent Res. 1982;90(6):417-22.
20. Ramfjord SP. Indices for Prevalence and Incidence of Periodontal Disease. The Journal of Periodontology. 1959;30(1):51-9.
21. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand. 1964;22:121-35.
22. Axell T, Mornstad H, Sundstrom B. The relation of the clinical picture to the histopathology of snuff dipper"s lesions in a Swedish population. J Oral Pathol. 1976;5(4):229-36.
23. NIH National Cancer Institute Cancer Therapy Evaluation Program. Common terminology criteria for adverse events, CTCAE v5.0 Ctep.cancer.gov. November 27, 2017 [Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm.
24. The World Medical Association. Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Wma.net. July 9, 2018. [Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.
25. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH E6(R2) Guideline for Good Clinical Practice. Ema.Europa.eu. July 1, 2002. Last updated December 15, 2016. [Available from: https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice.
26. European Commission. Clinical Trials – Directive 2001/20/EC. Ec.Europa.eu.April 4, 2001. [Available from: https://ec.europa.eu/health/medicinal-products/clinical-trials/clinical-trials-directive-200120ec_en.
27. European Commission. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). eur-lex-europa.eu.2016 [Available from: https://eur-lex.europa.eu/eli/reg/2016/679/oj.

## 19 SIGNATURES

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]