Clinical Study Protocol	
Investigational products	Dry nicotine pouches
Sponsor study code	SM25-02
Protocol Version and Date	Final v1.1; 09OCT2025

Risk Assessment of Dental Plaque Acidogenicity Following Short-Term Exposure to Nicotine Pouches [redacted]

Test products and doses

- Nicotine pouch (NP) Dry 3 mg
- NP Dry prototype A 3 mg [redacted]
- NP Dry prototype B 3 mg [redacted]

Sponsor signatory

[redacted]

Swedish Match North Europe AB
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Principal Investigator

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Clinical study conducts and management

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1 STUDY SYNOPSIS

Study title

Risk Assessment of Dental Plaque Acidogenicity Following Short-Term Exposure to Nicotine Pouches [redacted]

Study code	**Planned study period**
SM25-02	Q4 2025 to Q1 2026

Principal Investigator

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Study design

This is an open-label, randomized, 3-way crossover, single administration study, designed to assess the effects on dental plaque acidogenicity after using Dry 3 mg nicotine pouches (NPs), [redacted] in current daily users of NP or tobacco-based snus.

Objectives

Primary objective

To compare the effects on dental plaque acidogenicity after short-term exposure to three different Dry 3 mg NPs [redacted]

Secondary objectives

1. To compare the effects on buffer capacity after short-term exposure to three different Dry 3 mg NPs [redacted]
2. To compare the effects on the oral mucosa after short-term exposure to three different Dry 3 mg NPs [redacted]
3. To compare the subjectively perceived salivary stimulation effect after short-term exposure to the three different Dry 3 mg NPs [redacted]
4. To evaluate the safety and tolerability of the three different Dry 3 mg NPs, [redacted] in current daily users of NPs or tobacco-based snus.

Exploratory objective

To compare the effects on pH in unstimulated saliva after short-term exposure to three different Dry 3 mg NPs [redacted]

Endpoints

Primary endpoint

The difference in dental plaque acidogenicity, assessed by measuring pH in plaques, after short-term exposure (45 minutes) to three different Dry 3 mg NPs [redacted]

Secondary endpoints

1. The difference in salivary buffer capacity, assessed in unstimulated saliva, after short-term exposure (45 minutes) to three different Dry 3 mg NPs [redacted]

2. The difference in the effects on the oral mucosa, in the form of redness and mechanical irritation, after short-term exposure (45 minutes) to three different Dry 3 mg NPs [redacted]
3. The difference in the subjectively perceived salivary stimulation effect, assessed using a 100 mm visual analogue scale (VAS), after short-term exposure (45 minutes) to the three different Dry 3 mg NPs [redacted]
4. Frequency, intensity, and seriousness of adverse events (AEs).

Exploratory endpoint

The difference in pH levels in unstimulated saliva, assessed using a pH meter, after short-term exposure (45 minutes) to three different Dry 3 mg NPs [redacted]

Number of subjects planned

Approximately 24 subjects will be screened with the aim of achieving 12 randomized and fully evaluable subjects. A fully evaluable subject is defined as one who has used the investigational products (IPs) and completed all study visits. An effort will be made to randomize at least 3 female subjects (25%).

Diagnosis and main eligibility criteria

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

Women 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, 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 test laboratory for a screening visit followed by three usage visits on separate days. The screening (Visit 1) will take place within 4 weeks prior to the first usage visit and will involve an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, a brief oral examination, urine pregnancy testing (for women of childbearing potential only), and collection of medical history. Additionally, the salivary secretion rate, buffer capacity, and the number of cariogenic bacteria (*Streptococcus mutans* and lactobacilli) will be assessed.

Before each usage visit (Visits 2-4), scheduled at the same time of day for each subject, the subjects will refrain from approximal tooth cleaning for 24 hours and toothbrushing for 12 hours prior to the visits. Additionally, subjects will be instructed not to eat, drink, chew gum, use nicotine-containing products (including smoking), or engage in any other mouth-related procedures 1 hour before the visits. Subjects will use their regular toothbrush, which may be manual or electric, throughout the study. Urinary pregnancy tests may be conducted at Visits 2-4 for women of childbearing potential.

On the first usage visit (Visit 2), subjects will be randomized to one of three usage sequences. During each usage visit (Visits 2-4), the subject will place the IP in their upper vestibulum, where they normally place their pouches, using their fingers. The IP will be kept in place for 45 minutes. Plaque acidogenicity will be assessed by measuring pH in the plaque at four different locations before placement (baseline) and up to 45 minutes afterwards. All pH assessments will be performed on the same side as the IP placement.

The salivary buffer capacity will be assessed at baseline (0 minutes), and at 15, 30, and 45 minutes. Unstimulated saliva will be collected at each time point.

The effects on the oral mucosa from the usage of each IP will be assessed before (baseline) and after 45 minutes of usage. The effects in the form of redness and mechanical irritation will be graded as: no effect, mild effect, and severe effect. Photos will be taken before and after usage of each IP to enable a more detailed comparison of the effects.

The subjective salivary stimulation effect of each IP will be evaluated after 30 minutes of use using a 100 mm VAS, anchored with "no effect" to "extremely large effect".

AEs will be collected by subject interview and those spontaneously reported by the subjects from the first usage visit (Visit 2) to the last usage visit (Visit 4).

Investigational products (IPs) and dosage

IP	Nicotine content
NP Dry	3 mg nicotine/pouch
NP Dry prototype A [redacted]	3 mg nicotine/pouch
NP Dry prototype B [redacted]	3 mg nicotine/pouch

Duration of treatment

Each IP pouch will be used for 45 minutes.

Duration of each subject's involvement in the study

The participants will visit the test laboratory on four occasions, with at least three days between each visit, and each visit will last for approximately 1.5 hours. Each subject is therefore expected to participate in the study for approximately two weeks, excluding the screening period.

Assessment of dental plaque acidogenicity

Plaque acidogenicity will be assessed by measuring pH in the plaque before placement (baseline) and at 2, 5, 10, 15, 20, 30, and 45 minutes afterwards. A microelectrode (Beetrode) or a strip, the latter in case of difficulties in acquiring microelectrodes, will be placed at four different locations: 1) immediately mesial to the pouch, 2) immediately distal to the pouch, 3) interproximal between the front tooth/canine (tooth 13 and 12 or tooth 22 and 23), and 4) interproximal between the premolar/molar (tooth 16 and 15 or tooth 25 and 26). All pH assessments will consequently be performed on the same side as the IP placement.

Assessment of salivary secretion rate, buffer capacity, and number of cariogenic microorganisms

The salivary secretion rate will be assessed by collecting passively dropping saliva (unstimulated saliva).

The salivary buffer capacity will be assessed during the use of the IPs at baseline (before usage), and at 15, 30, and 45 minutes, with unstimulated saliva collected at each time point. Additionally, the pH will be measured using a pH meter.

At screening, collected saliva will be plated on various agar plates to quantify colony-forming units of *Streptococcus mutans* and lactobacilli.

Assessment of effects on the oral mucosa

The effects on the oral mucosa from the usage of each IP will be assessed before (baseline) and after 45 minutes of usage. The effects in the form of redness and mechanical irritation will be graded as: no effect, mild effect, and severe effect. Photos will be taken before and after usage of each IP to enable a more detailed comparison of the effects.

Assessment of subjective salivary stimulated effect

The subjective salivary stimulated effect of the product will be evaluated after 30 minutes of use using a 100 mm VAS, anchored with “no effect” to “extremely large effect”.
Safety assessment AEs will be collected by subject interviews and those spontaneously reported by the subjects from the first usage visit (Visit 2) to the last usage visit (Visit 4).
Statistical methods No formal sample size calculation has been performed. Based on previous clinical studies using the method for dental plaque acidogenicity (Studies SM17-02 and SM22-01), a total of 12 subjects is considered sufficient to generate data that can detect a clinically significant increase in plaque acidogenicity for the two prototype products versus the reference product NP Dry 3 mg. Descriptive analysis will be performed to calculate the mean ± standard deviation. Statistical analyses between the different products will be conducted for the various variables using two-way ANOVA followed by Tukey's HSD test. The relationship between the different collected variables will be analyzed using Pearson correlation coefficient. All analyses will be performed using PRISM 8 (GraphPad Software, San Diego, CA, US). A p-value < 0.05 is considered statistically significant. No adjustment for multiple comparisons will be made. No imputation of missing data will be performed.
Study reporting 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.

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
ADL	Activities of daily living
AE	Adverse event (or adverse experience)
CRF	Case report form
CSP	Clinical study protocol
CSR	Clinical study report
CTCAE	Common terminology criteria for adverse events
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
NP	Nicotine pouch
PI	Principal Investigator
QC	Quality control
SAE	Serious adverse event
SD	Standard deviation
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 AB
Maria Skolgata 83
SE-118 53 Stockholm, Sweden

Sponsor's medical representative

[redacted]

Sponsor's project manager

[redacted]

Sponsor´s medical writer

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Clinical conduct and management

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Investigational product manufacturing Swedish Match

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Swedish snus is a tobacco-based product that has been used for a long time in Sweden. No increased risk of caries is associated with snus use, and no difference in the number of filled teeth has been found when comparing matched adolescent Swedish 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 moist snuff and other smokeless products, which have been found to increase the risk of dental caries (3, 4).

Today, there are several different types of smokeless nicotine-containing products on the market, with the same use topography and systemic nicotine exposure as snus. These products can be divided into tobacco- and non-tobacco-leaf-containing products. In recent years, the market for non-tobacco-leaf-containing products, such as nicotine pouches (NPs), has grown tremendously. ZYN, one of several NP brand names, is available in two product types: Dry and Moist.

Previous clinical oral health studies (5, 6) (SM17-02 and SM22-01) found that ZYN products did not adversely affect dental plaque acidogenicity, whether after single-dose administration (5) or after four weeks of replacing tobacco-based snus with ZYN (6).

As ZYN Dry products have a low moisture content (approximately 2-3%), they typically need to be moistened by saliva for some time before the flavor becomes noticeable, which some consumers may perceive negatively. [redacted] However, it is not known whether this modification of the pouch paper would affect dental plaque acidogenicity and other variables.

6.2 Study rationale

Tobacco harm reduction involves adopting strategies to minimize the health risks associated with tobacco use, particularly for individuals who do not wish to quit nicotine usage (7). This approach includes transitioning from more harmful combustible cigarettes to potentially less harmful alternatives such as Swedish snus and NPs, providing viable options for nicotine delivery with potentially reduced health risks.

As mentioned above, ZYN Dry products have a low moisture content and need to be moistened by saliva before the flavor becomes noticeable, which some consumers may perceive negatively. [redacted] how this modification affects dental plaque acidogenicity and other variables.

The overarching aim of the study is to clinically assess the effects on dental plaque acidogenicity and other variables after using Dry 3 mg NPs, [redacted] in current daily users of NPs or tobacco-based snus. The rationale for the study design is presented in Section 8.2.

6.3 Risk/benefit assessment

6.3.1 *Risk assessment*

All subjects must be daily users of NPs or tobacco-based snus for at least one year, consuming at least three cans per week. Consequently, the subjects are well acquainted with and accustomed to the effects of nicotine, minimizing the risk of developing any new nicotine dependency. The NP products contain 3 mg nicotine, and there are commercially available products with substantially higher nicotine content on the market.

Moreover, the nicotine in the Dry 3 mg NP products is of pharmaceutical grade, the 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 Dry 3 mg NP products are approved for use in food. Previous clinical studies with similar products have reported no AEs other than those likely attributed to nicotine exposure, such as salivation, nausea, and dyspepsia.

Subjects who intend to change their nicotine consumption habits or stop using nicotine-containing products, as well as those who are pregnant, breastfeeding, or planning to become pregnant during the study, are excluded from participation. Additionally, subjects with a history or presence of diagnosed hypertension, cardiovascular disease, or other medical conditions that make them particularly vulnerable to nicotine exposure are also excluded. Moreover, individuals with below-average saliva production will be excluded due to the potential for AEs related to xerostomia, which could bias the study outcomes. However, it is expected that few, if any, subjects will have issues related to xerostomia, as all subjects will be current daily snus users for more than one year.

Aside from the risks related to the IPs, as detailed above, there may also be risks related to study-specific procedures. The assessments of dental plaque acidogenicity require participants to refrain from approximal tooth cleaning for 24 hours and toothbrushing for 12 hours prior to each of the three visits. 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 does not adversely affect dental health. Additionally, 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 due to their limited duration.

The PI at the test laboratory will ensure that adequate facilities and procedures are available to handle emergency situations should they occur during the study.

From a research ethics perspective, the potential AEs of the study procedures, which are likely to be minor and clinically insignificant, are counterbalanced by the reduced harm and risk of tobacco-related diseases when using NPs, especially compared to combustible cigarettes.

6.3.2 *Benefit assessment*

In analogy with a regular phase I study in healthy volunteers, there is no direct benefit for the subjects to participate in the study, aside from a brief oral examination, which may provide them with information on their general state of oral health.

6.3.3 *Risk/benefit conclusions*

NPs serve as substitutes for both combusted and non-combusted tobacco/nicotine-containing inhalation products (*e.g.*, conventional cigarettes, heated tobacco vaporizers, electronic cigarettes) and oral tobacco products (*e.g.*, tobacco-based snus and moist snuff). It is reasonable to assume that NP products will have less harmful effects on oral health than tobacco-based snus products, and even more so than combustible cigarettes. From this perspective, the aim of the present study aligns with society's overall goal of reducing the harm caused by tobacco use.

Overall, the potential AEs and risks associated with the study procedures are likely to be minor and clinically insignificant. The planned study assessments are sufficient to meet the scientific and medical goals. Therefore, the potential benefits of the study outweigh the potential risks for the daily habitual NP or tobacco-based snus users who will participate in this study

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective

To compare the effects on dental plaque acidogenicity after short-term exposure to three different Dry 3 mg NPs[redacted].

7.1.1 *Primary endpoint*

The difference in dental plaque acidogenicity, assessed by measuring pH in plaques, after short-term exposure (45 minutes) to three different Dry 3 mg NPs[redacted]

7.2 Secondary objectives

1. To compare the effects on buffer capacity after short-term exposure to three different Dry 3 mg NPs[redacted]
2. To compare the effects on the oral mucosa after short-term exposure to three different Dry 3 mg NPs[redacted]
3. To compare the subjectively perceived salivary stimulation effect after short-term exposure to the three different Dry 3 mg NPs[redacted]
4. To evaluate the safety and tolerability of the three different Dry 3 mg NPs, [redacted] in current daily users of NPs and/or tobacco-based snus.

7.2.1 *Secondary endpoints*

1. The difference in salivary buffer capacity, assessed in unstimulated saliva, after short-term exposure (45 minutes) to three different Dry 3 mg NPs[redacted]
2. The difference in the effects on the oral mucosa, in the form of redness and mechanical irritation, after short-term exposure (45 minutes) to three different Dry 3 mg NPs[redacted]
3. The difference in the subjectively perceived salivary stimulation effect, assessed using a 100 mm visual analogue scale, after short-term exposure (45 minutes) to the three different Dry 3 mg NPs[redacted]
4. Frequency, intensity, and seriousness of AEs.

7.3 Exploratory objective

To compare the effects on pH in unstimulated saliva after short-term exposure to three different Dry 3 mg NPs[redacted]

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

7.3.1 *Exploratory endpoint*

The difference in pH levels in unstimulated saliva, assessed using a pH meter, after short-term exposure (45 minutes) to three different Dry 3 mg NPs [redacted]

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This is an open-label, randomized, 3-way crossover, single administration study designed to assess the effects on dental plaque acidogenicity after using Dry 3 mg NPs, [redacted] in current daily users of NP or tobacco-based snus. The IPs are one Dry 3 mg NP (reference product) and two prototype products [redacted].

The study will include 12 randomized and fully evaluable subjects. Healthy male and female subjects aged ≥21-≤55 years who have used NP or tobacco-based snus for ≥1 year, with a minimum weekly consumption of 3 cans, may be eligible for participation. An effort will be made to randomize at least 3 female subjects (25%).

The subjects will visit the test laboratory on four occasions, with at least three days between each visit. Each visit will last approximately 1.5 hours. Consequently, each subject is expected to participate in the study for about two weeks, excluding the screening period.

The subjects will report to the test laboratory for a screening visit (Visit 1), which will take place within four weeks prior to the first usage visit (Visit 2). This screening visit will involve an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, a brief oral examination, urine pregnancy testing (for women of childbearing potential only), and collection of medical history. Additionally, the salivary secretion rate, buffer capacity, and the number of cariogenic bacteria (*Streptococcus mutans* and lactobacilli) will be assessed.

Before each usage visit (Visits 2-4), scheduled at the same time of day for each subject, the subjects will refrain from approximal tooth cleaning for 24 hours and toothbrushing for 12 hours prior to the visits. Additionally, subjects will be instructed not to eat, drink, chew gum, use nicotine-containing products (including smoking), or engage in any other mouth-related procedures 1 hour before the visits. Subjects will use their regular toothbrush, which may be manual or electric, throughout the study. Urinary pregnancy tests may be conducted at Visits 2-4 for women of childbearing potential.

On the first usage visit (Visit 2), subjects will be randomized to one of three usage sequences. During each usage visit (Visits 2-4), the subjects will place the IP in their upper vestibulum, where they normally place their pouches, using their fingers. The IP will be kept in place for 45 minutes. Dental plaque acidogenicity will be assessed by measuring pH in the plaque at four different locations before placement (baseline), and at 2, 5, 10, 15, 20, 30, and 45 minutes afterwards. All pH assessments will be performed on the same side as the IP placement.

The salivary buffer capacity will be assessed at baseline (0 minutes), and at 15, 30, and 45 minutes. Unstimulated saliva will be collected at each time point.

The effects on the oral mucosa from the usage of each IP will be assessed before (baseline) and after 45 minutes of usage. The effects, in the form of redness and mechanical irritation, will be graded as: no effect, mild effect, and severe effect. Photos will be taken before and after the usage of each IP to enable a more detailed comparison on the effects.

The subjective salivary stimulation effect of each IP will be evaluated after 30 minutes of use using a 100 mm VAS, anchored with “no effect” to “extremely large effect”.

AEs will be collected by subject interview and those spontaneously reported by the subjects from the first usage visit (Visit 2) to the last usage visit (Visit 4).

See Table 8.1-1 for the schedule of events applicable for each visit. Study assessments are described in Section 11.

Table 8.1-1 Schedule of events

Events	Visit 1 Screening	Visit 2 First usage visit	Visits 3-4 Second and third usage visit
Informed consent	X		
Demographics	X		
Medical/surgical history	X		
History of nicotine use	X		
Inclusion/exclusion criteria	X	X[1]	
Brief oral examination	X	X	X
Salivary secretion rate	X		
Salivary buffer capacity	X	X	X
Pregnancy test[2]	X	X[3]	X[3]
Baseline symptoms	X	X	
Prior and concomitant medications	X		
Randomization		X	
Administration of IP		X	X
Plaque acidogenicity[4]		X	X
Oral mucosa effects		X	X
VAS question		X	X
Collection of saliva		X	X
AEs		X	X

[1] Confirmation of eligibility criteria.
[2] Only women of childbearing potential.
[3] Only at the discretion of the Investigator on Visits 2-4.
[4] Assessed at 0, 2, 5, 10, 15, 30, and 45 minutes.

8.2 Rationale for study design

This is an open-label, randomized, 3-way crossover, single administration study designed to assess the effects on dental plaque acidogenicity after using Dry 3 mg NPs, [redacted] in current daily users of NP or tobacco-based snus.

Randomization will be used to minimize bias in the assignment of subjects to an IP use sequence, increasing the likelihood that known and unknown subject attributes (*e.g.*, demographic and baseline characteristics) will be evenly balanced.

A crossover design was chosen to yield a more efficient evaluation of NPs than a parallel study design, as fewer subjects are required since each subject will serve as their own control. To avoid carry-over effects, subjects will abstain from nicotine products (including smoking) for at least 1 hour prior to each IP use visit (Visits 2-4). Additionally, there will be at least three days between each of the three usage visits with single administration to allow for accurate assessment of dental plaque acidogenicity.

9 STUDY POPULATION

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

9.1 Recruitment

Participants will be recruited through locally distributed posters, targeting personnel and students at the Institute of Odontology in Gothenburg, Sweden. The recruitment materials will be created using advertising texts approved by the independent ethics committee (IEC).

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 with the informed consent process at the screening visit (Visit 1). The screening number will be manually assigned and recorded in the case report form (CRF). This 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

Approximately 20 subjects will be screened with the aim of achieving 12 randomized and fully evaluable subjects. A fully evaluable subject is defined as one who has used the IPs and completed all study visits. An effort will be made to randomize at least 3 female subjects (25%).

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

9.4 Inclusion criteria

For inclusion in the study, the 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 NPs or tobacco-based snus for ≥1 year, with a minimum weekly consumption of 3 cans.
3. Healthy male or female subject aged ≥21 to ≤55 years.
4. Normal stimulated salivary secretion rate (>0.7 mL/min).
5. At least 24 own teeth remaining and overall good oral health, as judged by the Investigator.
6. Women of child-bearing potential must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) or must agree to use a highly effective method of contraception with a failure rate of

<1% to prevent pregnancy for the duration of the study. The following are considered highly effective methods of contraception:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal),
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable),
- intrauterine device or intrauterine hormone-releasing system.

9.5 Exclusion criteria

Subjects must not enter 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 soft tissues (apart from oral mucosal lesions related to the use of snus), or extensive prosthetic work (*e.g.,* several implants, partial denture, dental veneers).
6. Any planned major surgery within the duration of the study.
7. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse, as judged by the Investigator.
8. Antibiotic use ≤4 weeks prior to the screening period.
9. Subjects who intend to change their nicotine consumption habit, including the intention to stop using nicotine products, within the next 3 months of the screening visit, as judged by the Investigator.
10. Subjects undergoing other dental treatment during the study period.
11. The Investigator considers the subject unlikely to comply with study procedures, restrictions, and requirements.

9.6 Restrictions during the study

Subjects must be willing to comply with the restrictions as outlined in Sections 9.6.1 and 9.6.2.

9.6.1 *General restrictions*

1. Tooth cleaning and toothbrushing: Subjects shall refrain from approximal tooth cleaning for 24 hours and toothbrushing for 12 hours prior to the usage visits (Visits 2-4).
2. Contraception requirements: Women of childbearing potential are expected to use contraceptive methods in accordance with inclusion criterion #6 or practice abstinence from heterosexual intercourse (if this is their consistent practice) during the study.
3. Tobacco/nicotine products: Subjects shall abstain from all other nicotine products 1 hour prior to the usage visits (Visits 2-4).
4. Mouth-related procedures: Subjects shall abstain from eating, drinking, or conducting any other mouth-related procedure for 1 hour prior to IP use, during IP use, and for 30 min after IP removal (Visits 2-4).
5. Participation in other clinical studies: Subjects are not allowed to participate in any other clinical studies or use antibiotics during the study period (Visits 1-4).

9.6.2 *Prior and concomitant therapy*

The use of antibiotics is not allowed from the screening visit (Visit 1) to the last usage visit (Visit 4) in accordance with exclusion criterion #8. Subjects undergoing other dental treatment during the study period will be excluded from the study according to exclusion criterion #10. There will be no other restrictions concerning concomitant medications or therapies, as long as the subject is on a stable course of medication from the screening visit to the last usage visit (Visit 4). 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 do not fulfill all eligibility criteria and are not subsequently included in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

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)
- Reserve subjects

For subjects who are re-screened, a new screening number will be assigned and a new, signed ICF must 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 can include:

1. AE (as judged by the Investigator and/or Sponsor)
2. Logistical problem
3. Lost to follow-up
4. Non-compliance with study schedule and restrictions
5. Pregnancy
6. Withdrawal of consent
7. Other

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

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. Any ongoing AEs will be followed up as described in Section 11.6.10.

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

9.8.3 *Subject replacement*

Subjects who are prematurely withdrawn prior to the start of IP use may be replaced at the discretion of the Sponsor.

9.9 Randomization

At Visit 2, subjects will be randomized to one of 3 use sequences. The following sequences will be randomized:

Sequence 1: A B C

Sequence 2: B C A

Sequence 3: C A B

Where

A = Dry 3 mg NP (reference)

B = Prototype A Dry 3 mg NP [redacted]

C = Prototype B Dry 3 mg NP [redacted]

As this is an open-label study, the IP use sequence to which each subject is allocated will be recorded in the CRF.

10 INVESTIGATIONAL PRODUCTS

The IPs are supplied by Swedish Match.

10.1 Identity of investigational products

The IPs that will be used in the study are detailed in Table 10.1-1.

Table 10.1-1 Identity of IPs and nicotine contents

IP	Nicotine content
NP Dry	3 mg nicotine/pouch
NP Dry prototype A [redacted]	3 mg nicotine/pouch
NP Dry prototype B [redacted]	3 mg nicotine/pouch

10.2 Manufacturing, packaging, and labeling

All IPs are manufactured and packaged by Swedish Match in compliance with Swedish food production laws. Production sites and batch IDs for the IPs will be documented in the trial master file (TMF).

IPs will be transferred from the original container and individually packaged in identical sealed, food-approved test containers at the Swedish Match analytical laboratory. The containers will be labeled with unique identification numbers by Swedish Match in accordance with the randomization list. IPs will be shipped by Swedish Match directly to the test laboratory (Department of Cariology, Institute of Odontology, 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 dispensed according to the randomization list by the site personnel. The Investigator will maintain a storage and accountability log, as well as a dispensing log, detailing the dates and quantities of study IPs received and used by each subject, as well as any IPs destroyed at the end of the study.

10.5 Investigational product use instructions

A single pouch will be administered during each usage visit (Visits 2-4). The subject will place the IP in their upper vestibulum, where they normally place their pouches, using their fingers. The IP will be kept in place for 45 minutes.

10.6 Investigation product final accountability

Any unused IP will be destroyed and documented at the test laboratory upon confirmation from the Sponsor.

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 (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 CRF and in all required reports.

The different study assessments will be collected in the following order:

1. Dental plaque acidogenicity
2. Salivary buffer capacity
3. Effects on oral mucosa
4. Subjectively perceived salivary stimulation
5. Collection of saliva

Subjects will be scheduled for three usage visits (Visits 2-4), with at least three days between each visit. For plaque acidogenicity, assessments will be performed before placement of the IP and at 2, 5, 10, 15, 20, 30, and 45 minutes afterwards. Allowed deviations for these time points are ±1 minutes for the time points 0-15 minutes and ±2 minutes for the time points 20-45 minutes. For assessments of salivary buffer capacity, effects on the oral mucosa, and subjectively perceived salivary stimulation, allowed deviations will be ±2 minutes.

11.2 Demographics and other baseline characteristics

11.2.1 *Informed consent*

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

11.2.2 *Demographic information*

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

11.2.3 *Medical/surgical history*

Medical/surgical history will be obtained through subject interviews to verify that the eligibility criteria are met.

11.2.4 *History of nicotine use*

The history of nicotine use, including brands, average daily consumption over the past 30 days, duration of use (years), and smoking history (cigarettes and e-cigarettes), will be obtained thorough subject interviews.

11.2.5 *Eligibility criteria*

Eligibility criteria must be checked during screening and verified before randomization at Visit 2. The criteria are specified in Sections 9.4 and 9.5.

11.2.6 *Oral examination*

At screening (Visit 1), a brief oral examination will be performed to assess overall oral health status, normal stimulated salivary secretion rate (>0.7 mL/min), the presence of at least 24 natural teeth, and to quantify cariogenic bacteria (*Streptococcus mutans* and lactobacilli). A brief oral examination will also be conducted at each usage visit to ensure continued good oral health status.

11.2.7 *Pregnancy test*

Women of childbearing potential will undergo a urine pregnancy test at screening (Visit 1) and at the discretion of the Investigator during the usage visits (Visits 2-4).

11.2.8 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF and the start of the randomization at the second visit (*i.e.,* an event that occurs while the subjects are using their regular nicotine product). Such events are not considered AEs and will be recorded as baseline symptoms in the Medical History Log in the CRF.

11.2.9 *Prior and concomitant medication*

Prior and concomitant medications taken within 2 weeks prior to screening will be documented through subject interviews to assess the subject's current medication status.

Medications will be classified as prior if the stop date was before or on the day of randomization, prior to the start of IP use, and as concomitant if ongoing on the day of IP use visit (Visit 2), or if they started or stopped after the assessments on that visit. To distinguish between prior and concomitant medications on the second visit (Visit 2), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the CRF.

Any use of concomitant medication from screening until the last usage visit (Visit 4) will be documented in the subject's CRF. 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 CRF.

11.3 Assessment related to the primary endpoint

11.3.1 *Dental plaque acidogenicity*

An iridium microelectrode (Beetrode) will be inserted into the plaque at four different locations: 1) immediately mesial to the pouch, 2) immediately distal to the pouch, 3) interproximal between the front tooth/canine (tooth 13 and 12 or tooth 22 and 23), and 4) interproximal between the premolar/molar (tooth 16 and 15 or tooth 25 and 26). The electrode will be connected to a pH meter (Orion SA720 pH/ISE Meter) along with a reference electrode. The reference electrode will be placed into a solution of 3 M KCl with the subject´s finger placed in the solution to create a salt bridge.

After calibration and baseline assessment (0 minutes), the subjects will place the IP in their mouth and use it for 45 minutes. The pH will then be measured at 2, 5, 10, 15, 20, 30, and 45 minutes on the same side as the IP placement. In case of difficulties in acquiring microelectrodes, the strip method will be used; a paper indicator strip will be inserted in the area and kept in place for 10 seconds, 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 CRF.

11.4 Assessments related to secondary endpoints

11.4.1 *Buffer capacity*

The buffering capacity of saliva will be assessed at baseline (0 minutes), before use of the IP, and at 15, 30, and 45 minutes of usage. Unstimulated saliva, passively dropping saliva, will be collected at each time point. Then, 3 mL of 0.001 M HCl and 1 drop of octyl alcohol will be added to 1 mL of saliva. The sample will be aerated for 20 minutes, after which the final pH will be measured. The date and time of collection of each sample and the assessed pH levels will be recorded in the CRF.

11.4.2 *Effects on oral mucosa*

The effects on the oral mucosa will be assessed before use of the IP and after 45 minutes of usage. The effects, in the form of redness and mechanical irritation, will visually be graded as: no effect, mild effect, and severe effect. Photos will be taken before and after usage of each IP to enable a more detailed comparison of the effects. The date and time of visual examination and assessed grading will be recorded in the CRF.

11.4.3 *Subjectively perceived salivary stimulation*

The subjective salivary stimulation effect of the IP will be evaluated after 30 minutes of use using a 100 mm VAS, anchored with “no effect” to “extremely large effect”. Subjects will mark their rating with a line on the scale. The date and time of the rating, along with the assessed scores, will be recorded in the CRF.

11.5 Assessments related to exploratory endpoint

11.5.1 *pH level in saliva*

Unstimulated saliva will be collected passively to assess pH levels before and after IP usage using a pH meter. The date and time of collection, along with the assessed pH levels, will be recorded in the CRF.

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

11.6.2 *Definition of serious adverse event*

An SAE is any AE that:

- results in death,
- is life-threatening (the subject was at risk of death at the time of the reaction, hypothetically),
- 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) (may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or require intervention to prevent other outcomes).

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

Planned hospitalizations or surgical interventions for pre-existing conditions that did not change in intensity are not SAEs.

If there is any doubt 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 through subject interviews from the start of randomization at Visit 2 until the last usage visit (Visit 4).

Any AE starting on the day of the randomization must be recorded with the start time.

At Visit 4, 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 study concludes. However, if an Investigator learns of any SAE, including death, at any time after a participant has been discharged from the study, and considers the event reasonably related to the study intervention or 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 (8). Grade refers to the severity of the AE, with CTCAE displaying Grades 1 through 5, each with unique clinical descriptions based on this general guideline.

The Investigator must assess AE intensity using the definitions in Table 11.6-1 and record it on the AE log in the CRF.

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 in the AE log of the CRF.

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 in Table 11.6-3 and record it in the AE log of the CRF.

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:

- spontaneously reported by the subject
- observed by the Investigator or medical personnel
- 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 CRF. The Investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates and times; intensity; causal relationship to IP; action taken; and outcome.

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

AEs must be recorded individually, except when they are 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 hours of awareness to the Sponsor and PMI Safety. All available information regarding the SAE should be entered in the AE log for the specific subject. The SAE report is reviewed by the Sponsor and PMI Safety to ensure it is valid and correct. For fatal or life-threatening SAEs where important or relevant information is missing, immediate follow-up is undertaken. Investigators or other site personnel should inform the Sponsor and PMI Safety of any follow-up information on a previously reported SAE immediately, but no later than at the end of the next business day after becoming aware of it.

The SAE should be reported by manually completing the paper SAE Form provided in the Investigator site file. The completed, signed, and dated paper SAE Form should be scanned and e-mailed within 24 hours to:

[redacted]

11.6.10 *Treatment and follow-up of adverse events*

Subjects with AEs during the study must be treated according to daily clinical practice at the Investigator´s discretion.

AEs must be followed up until resolution or the end of the study, whichever comes first. At each subject's end of study visit (Visit 4), 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 do not need to be followed up until resolution.

The Investigator is responsible for following up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and for reporting all relevant new information to the

Sponsor using the same procedures and timelines as the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultations.

11.6.11 *Procedures in case of pregnancy*

In case of pregnancy or suspicion of pregnancy, IP usage must be stopped immediately, and the subject discontinued from the study. Pregnancy itself is not regarded as an AE unless there is suspicion that the IP may have interfered with contraceptive effectiveness. 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 is discontinued from the study.

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

11.6.12 *Treatment of overdose*

Over-dosing is not applicable, as subjects will only be administered a single pouch during each usage visit. 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 employed in standard medical care and Phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collections

The procedures for collecting samples to assess dental plaque acidogenicity, buffer capacity, and pH levels in unstimulated saliva are described in Section 11.3.1.

12.2 Handling, storage, and destruction of laboratory samples

Saliva samples will be registered in 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.

Urine samples for pregnancy testing will be disposed of after analysis.

12.3 Chain of custody of biological samples

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

The Investigator ensures full traceability of collected biological samples from the subjects while they are in storage at the test laboratory.

12.4 Withdrawal of informed consent for donated biological samples

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

The PI will ensure that:

1. The subject´s withdrawal of informed consent is immediately notified to the Sponsor.
2. Biological samples from the subject, if stored at the research clinic or in the Biobank, will be promptly identified, disposed of or destroyed, and the action will be 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 the processes, systems (facilities, computerized systems), and data that are critical to ensuring human subject protection and the reliability of study results, in accordance with applicable SOPs and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6 (R2) (9).

13.2 Quality assurance and quality control

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

QC should be applied at 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 PI while 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 (10) and are consistent with the ICH E6(R2) guideline for GCP (9), the EU Clinical Trials Directive (11), and applicable local regulatory requirements.

14.2 Ethics and regulatory review

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

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

The Sponsor will provide the IEC and 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 provide each potential study subject with adequate verbal and written information before any study-specific assessments are performed.

This information will include the objectives and procedures of the study, as well as any risks or inconveniences 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 both the subject and 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 CRF. 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 (12).

The potential study subject will be informed that by signing the ICF, he/she approves that authorized representatives from the Sponsor 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. The request will be raised to the PI.

The Investigator must file a subject identification list that includes sufficient information to link records, such as the CRF and clinical records. This list should be preserved for possible future inspections/audits but must not be made available to the Sponsor except for auditing purposes.

Personal data collected in the study, such as health information, is considered sensitive personal data. This data will be pseudonymized, meaning personal data 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, Swedish Match North Europe AB and the PI, are joint data controllers for the collection and overall processing of personal data. However, the PI is solely responsible for the processing of personal data related to medical record-keeping, sampling, health laboratory analyses, and biobanking.

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 test laboratory, including source data verification. The purpose of an audit is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the CSP, 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. The University of Gothenburg has a company insurance covering services performed at the test laboratory.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Prior to the enrolment of the first study subject, the PI will ensure that all study personnel are adequately trained and familiar with the CSP, related documents, and any study-specific procedures or systems.

Given the PI´s prior experience with similar studies and familiarity with the IP, a formal study initiation visit will not be conducted. Instead, the Sponsor will ensure that all necessary study materials and documentation are provided, and available to support the site as needed.

The PI will ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have detailed knowledge of and training in the procedures they are to execute. Any new information relevant to the performance of this study must be communicated to the staff involved in a timely manner.

The PI will maintain a list of all personnel involved in the study, including their roles and delegated responsibilities. A current *Curriculum Vitae* will be available for key staff to whom study-specific duties are delegated.

15.2 Clinical monitoring

No monitoring activities will be conducted during this study. All QC and safety measures will be managed through internal procedures and regular reviews by the study staff. Any deviations or issues will be documented and addressed according to the CSP and applicable guidelines.

15.3 Source data documents

Source documents are all documents used by the Investigator that relate to the subject's medical history, verify the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. They include laboratory notes, memoranda, material dispensing records, subject files, *etc*. The Investigator should guarantee access to source documents to 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 the Sponsor and the PI must be in place before any study-related procedures can take place or subjects can be enrolled.

15.5 Study timetable and end of study

The study is expected to start in Q4 2025 and be completed by Q1 2026.

A subject is considered to have completed the study if they have completed all usage visits in the study (Visits 2-4). Each subject who completes the study will participate for a period of 3 weeks, not including the preceding screening period.

The end of the study is defined as the last visit, *i.e.*, the last usage visit (Visit 4), 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 ensure 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 PI. The CSR will be reviewed and approved by, at a minimum, the PI 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 the finalization of the CSR. This includes any original source documents related to the study. It is the responsibility of the Sponsor to inform the PI 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 completed CRF is 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 database setup and management, data entry and verification, data validation, QC of the database, and documentation of performed activities, including information on discrepancies in the process. The database will be designed in accordance with the CSP.

16.1 The CRF

Clinical data will be entered into a paper CRF, directly from the source documents or the dental chair. Source data are to be defined before the 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 study personnel prior to the study being initiated.

16.2 The entering of data into the CRF

All written entries, corrections, and alterations in the CRF are to be made by the Investigator or designated site personnel. All data should be entered in English. The CRFs 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 CRF.

The Investigator must verify that all data entries in the CRFs are accurate and correct, and the PI will manually sign the CRFs.

16.3 Medical coding

The PI will handle AEs and medical/surgical history, and no formal medical coding will be performed. All documents will be approved by the Sponsor prior to database lock.

16.4 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.

17.1 General

Descriptive analysis will be performed to calculate the mean ± standard deviation. Statistical analysis between the different products will be conducted for the various variables using two-way ANOVA followed by Tukey´s HSD test. The relationship between the different collected variables will be analyzed using Pearson correlation coefficient. All analysis will be performed using PRISM 8 (GraphPad Software, San Diego, CA, US). A p-value <0.05 is considered statistically significant.

No adjustment for multiple comparisons will be made.

No imputation of missing data will be performed.

17.2 Determination of sample size

No formal sample size calculation has been performed. Based on previous clinical studies using the method for dental plaque acidogenicity (studies SM17-02 and SM22-01) (5, 6), a total of 12 subjects are considered sufficient to generate data that can detect a clinically significant increase in plaque acidogenicity for the two prototype products versus the reference product Dry 3 mg NP.

17.3 Analysis data set

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.

17.4 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 and listings.

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 *Usage compliance*

The number of subjects using the IPs will be presented by 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*

The primary endpoint is the difference in dental plaque acidogenicity, assessed by measuring pH in plaques after short-term exposure (45 minutes) to three different Dry 3 mg NPs. The assessment of dental plaque acidogenicity will be calculated at each time point and presented with summary statistics, including the average for all four sites and the average per site, as described in Section 17.1.

17.6 Analysis of secondary endpoints

17.6.1 *Buffer capacity*

The difference in salivary buffer capacity, assessed in unstimulated saliva after short-term exposure (45 minutes) to three different Dry 3 mg NPs, will be calculated at each time point and presented with summary statistics, as described in section 17.1.

17.6.2 *Effects on the oral mucosa*

The difference in the effects on the oral mucosa, in the form of redness and mechanical irritation, after short-term exposure (45 minutes) to three different Dry 3 mg NPs will be assessed using visual grading (no effect, mild effect, and severe effect) and clinical photographs. The clinical evaluation will be detailed in a descriptive text format.

17.6.3 *Subjectively perceived salivary stimulation*

The difference in the subjectively perceived salivary stimulation effect, assessed using a 100 mm VAS, after short-term exposure (45 minutes) to the three different Dry 3 mg NPs, will be presented through descriptive statistics.

17.6.4 *Analysis of adverse events*

All AEs, including SAEs, intensity, and deaths, will be listed by subject and IP.

17.7 Analysis of exploratory endpoint

17.7.1 *pH levels in saliva*

The difference in pH levels in unstimulated saliva, assessed using a pH meter, after short-term exposure (45 minutes) to three different Dry 3 mg NPs, will be calculated at each time point and presented with summary statistics, as described in Section 17.1.

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. 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.
6. Swedish Match Europe Division. ISRCTN13243849: Effects on oral health from the usage of ZYN Dry and ZYN Moist nicotine pouches. isrctn.com. Applied July 7, 2022. Last edited Dec 4, 2024. [Available from: https://www.isrctn.com/ISRCTN13243849.
7. Stratton K, Shetty P, Wallace R, Bondurant S. Clearing the smoke: the science base for tobacco harm reduction--executive summary. Tob Control. 2001;10(2):189-95.
8. 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.
9. 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.
10. 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/.
11. 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.
12. 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

19.1 Principal Investigator

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

Peter Lingström, DDS, PhD
Professor, Senior Dental Officer
Sahlgrenska Academy, University of Gothenburg

19.2 Sponsor's signatory

I, the undersigned, approve this CSP.

Mikael Staaf, PhD
Senior Manager Product Science

Swedish Match North Europe AB