Clinical Study Protocol	
Investigational products	ZYN Dry 6 mg
Sponsor study code	SM21-01
Protocol Version and Date	Final v1.0; 29OCT2021

Nicotine plasma concentrations, pharmacokinetics, and pharmacodynamics following single doses of flavored oral tobacco-derived nicotine pouches in current, daily oral tobacco/nicotine users

Test products and doses

ZYN Dry Virginia Blend 6 mg
ZYN Dry Kentucky Bold 6 mg
ZYN Dry Cherry 6 mg
ZYN Dry Dragonberry 6 mg
ZYN Dry Peach 6 mg
ZYN Dry Wine 6 mg
ZYN Dry Bourbon 6 mg
ZYN Dry Dark Rum 6 mg

Comparator product and dose

ZYN Dry Smooth 6 mg

Sponsor signatory

[redacted]

Swedish Match
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1 STUDY SYNOPSIS

Study title	
Nicotine plasma concentrations, pharmacokinetics, and pharmacodynamics following single doses of flavored oral tobacco-derived nicotine pouches in current, daily oral tobacco/nicotine users.	
Study code	**Planned study period**
SM21-01	Q1 2022 to Q2 2022
Principal Investigator	
Johan Nilsson, MD, PhD CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden	
Study design	
This is an open-label, randomized, 9-way cross-over, single dose administration study, designed to assess the impact of flavors on nicotine exposure. The investigational products (IPs) are oral tobacco-derived nicotine (OTDN) pouches, ZYN Dry 6 mg: including 1 unflavored product (ZYN Dry Smooth) and 8 flavored products (ZYN Dry Virginia Blend, Kentucky Bold, Cherry, Dragonberry, Peach, Wine, Bourbon and Dark Rum).	
Objectives Primary objective The primary objective of the study is to evaluate the impact of flavor on nicotine exposure, after the administration of single doses of unflavored ZYN Dry Smooth 6 mg and flavored ZYN Dry Virginia Blend 6 mg. Secondary objectives 1. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored and flavored ZYN Dry products. 2. To compare the pharmacokinetic (PK) profile between the unflavored and flavored ZYN Dry products. 3. To evaluate the impact of flavors on pharmacodynamic (PD) effects, measured as the pulse rate and subjective outcome measures, for all the ZYN Dry products. 4. To evaluate the safety and tolerability of ZYN Dry products in current, daily oral tobacco/nicotine users. Exploratory objectives 1. To evaluate the impact of flavors on "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice for all the ZYN Dry products. 2. To compare the nicotine extraction-normalized PK parameters area under the curve from 0 to infinity (AUC_{inf}) and maximum observed concentration (C_{max}) between the unflavored and flavored ZYN Dry products. The results of the exploratory objectives may not be reported in the clinical study report (CSR).	

Endpoints

Primary endpoint

PK of nicotine in plasma: The equivalence (90% confidence interval for the ratio between 0.8 and 1.25) in the baseline-adjusted AUC_{inf} based on nicotine plasma concentrations after the administration of single doses of unflavored ZYN Dry Smooth 6 mg and flavored ZYN Dry Virginia Blend 6 mg.

Secondary endpoints

1. The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored and flavored ZYN Dry products.

2a. PK of nicotine in plasma: The equivalence between the unflavored and flavored ZYN Dry products in the non-adjusted and baseline-adjusted PK parameters C_{max} and AUC_{inf} based on plasma concentrations of nicotine after the administration of single doses.

2b. PK of nicotine in plasma: The difference between the unflavored and flavored ZYN Dry products in the non-adjusted and baseline-adjusted PK parameters time of occurrence of C_{max} (T_{max}), the area under the curve from 0 to 1.5 h ($AUC_{0\text{-}1\ 5h}$), and the AUC from 0 to the last measurable time point ($AUC_{0\text{-}last}$).

3a. The difference between the unflavored and flavored ZYN Dry products for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

3b. The difference between the unflavored and flavored ZYN Dry products for the highest recorded increase (E_{max}) in the subjective parameters "craving" and "satisfaction", measured using a 100 mm visual analogue scale (VAS) after IP administration.

3c. The difference between the unflavored and flavored ZYN Dry products for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 60 min after IP administration.

4. Frequency, intensity, and seriousness of adverse events (AEs).

Exploratory endpoints

1. The difference in "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice between the unflavored and flavored ZYN Dry products, measured on a 3-point scale using a multiple-choice question (MCQ) 60 min after IP administration.

2. The difference in nicotine extraction-normalized AUC_{inf} and C_{max} between the unflavored and flavored ZYN Dry products.

Number of subjects planned

Approximately 63 subjects will be screened with the aim to achieve 42 randomized subjects and 36 fully evaluable subjects. A fully evaluable subject is defined as a subject who has received all 9 IPs and who has completed all study visits. An effort will be made to randomize at least 17 female subjects (approximately 40 %).

Diagnosis and main eligibility criteria

Healthy male or female subjects aged ≥21 years who have used oral tobacco/nicotine products for ≥1 year, with a minimum daily consumption of 5 or more pouches, who are willing and able to use brands with nicotine content ≥1 %, may be considered to be eligible for participation in the study. Subjects have to 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 that may interfere with the evaluation 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 9 treatment visits (Visit 2-10) on separate days. Screening (Visit 1) will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, a brief physical examination, laboratory tests, electrocardiogram (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI).

Prior to each treatment visit (Visit 2-10), subjects will abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for >12 h. All treatment sessions will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

The IPs will be administered as single pouches in a pre-determined randomized order. Subjects will keep the pouch still between the upper lip and gum for 60 min and will be instructed not to manipulate the pouch with the tongue or lips. They will also be instructed not to eat, drink, chew gum, or brush their teeth for 30 min before, during, or 30 min after IP administration.

After 60 min, each used pouch will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches from the same batch will serve as references and will be stored at -20 °C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-administration to 6 h after each IP administration. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using VAS questions) at the same pre-defined time points as well as an MCQ 60 min after IP administration.

AEs will be collected by subject interview from the start of IP administration (Visit 2) until the last treatment visit (Visit 10).

Investigational products (IPs) and dosage

IP	Nicotine content
ZYN Dry Smooth (unflavored comparator)	6 mg nicotine/pouch
ZYN Dry Virginia Blend	6 mg nicotine/pouch
ZYN Dry Kentucky Bold	6 mg nicotine/pouch
ZYN Dry Cherry	6 mg nicotine/pouch
ZYN Dry Dragonberry	6 mg nicotine/pouch
ZYN Dry Peach	6 mg nicotine/pouch
ZYN Dry Wine	6 mg nicotine/pouch
ZYN Dry Bourbon	6 mg nicotine/pouch
ZYN Dry Dark Rum	6 mg nicotine/pouch

1.

Duration of treatment

The participating subjects will receive IPs at 9 occasions, in a cross-over fashion, with 60 min of treatment per occasion.

Duration of each subject's involvement in the study

Each subject will participate in the study for a period of approximately 5 weeks, not including the preceding 4-week screening period.

Pharmacokinetic assessments

Blood samples for analysis of PK parameters will be collected pre-administration, and at 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 1h:15 min, 1 h:30 min, 2 h, 4 h and 6 h post-administration. The PK parameters in the study will include AUC_{inf}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}1\ 5h}$, C_{max}, T_{max} and $T_{1/2}$.

Pharmacodynamic assessments

The PD effects will be assessed by measuring pulse rate and subjective parameters (using VAS) from pre-administration (10 min prior to IP administration) to 6 h post-administration, at pre-defined time points. The subjective parameters that will be assessed are "craving", "satisfaction", "product-liking", and "intent to use again". In addition, "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice will be assessed using a 3-point scale questionnaire at 60 min post-administration.

Nicotine extraction assessment

Used pouches will be collected after 60 min of use for the determination of residual nicotine in the IPs. The extracted amount (mg/unit) and extracted fraction (%) of nicotine will be assessed.

Safety assessment

AEs will be collected by subject interview from the start of IP administration (Visit 2) until the last treatment visit (Visit 10).

Statistical methods

Using a coefficient of variation (CV) of 32.5%, based on previous studies, a power of 80% and a significance level of 10 %, 36 evaluable subjects will be needed. Assuming a dropout rate of 15%, 42 subjects will be randomized.

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]). In addition, for the parameters AUC and C_{max} the geometric mean and CV will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by IP, and by assessment time. Individual subject data will be listed by subject number, IP, 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 collection time point prior to each IP administration.

No adjustment for multiple comparisons will be performed. All formal comparisons will be made towards a designated reference product and all significant findings will be reviewed for medical relevance.

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.

2 TABLE OF CONTENTS

List of Tables

3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
ADHD	Attention deficit hyperactivity disorder
ADL	Activities of daily living
AE	Adverse event (or adverse experience)
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration *vs.* time curve
AUC_{inf}	AUC from 0 to infinity
$AUC_{0\text{-}last}$	AUC from 0 to time of last measurable concentration
$AUC_{0\text{-}t}$	AUC from 0 to the time t, *e.g.*, $AUC_{0\text{-}1.5h}$
BMI	Body mass index
C_{max}	Maximum observed concentration
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
DMP	Data management plan
eCRF	Electronic case report form
ECG	Electrocardiogram
EDC	Electronic data capture
EEA	European Economic Area
EMA	European Medicines Agency
E_{max}	Highest recorded change from baseline
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good clinical practice
GDPR	General data protection regulation
HIV	Human immunodeficiency virus
HR	Heart rate
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
$Lambda_z$	Estimate of the terminal elimination rate constant
LC-MS	Liquid chromatography-mass spectrometry
LLOQ	Lower limit of quantification
LSMeans	Least-squares means
MCQ	Multiple-choice question
MedDRA	Medical dictionary for regulatory activities
MPA	Medical Products Agency
MS	Mass spectrometry
NCA	Non-compartmental analysis
NRT	Nicotine replacement therapies
OTDN	Oral tobacco-derived nicotine
PD	Pharmacodynamic(s)
PI	Principal Investigator
PII	Personally Identifiable Information
PK	Pharmacokinetic(s)
PKAS	PK analysis set
PMTA	Premarket tobacco product application
PPE	Personal protective equipment
PR interval	(ECG) The time from the onset of the P wave to the start of the QRS complex
PT	Preferred term
PV	Pharmacovigilance
QA	Quality assurance
QC	Quality control
QRS interval	(ECG) The time required for a stimulus to spread through the heart's ventricles
QT interval	(ECG) The time from the beginning of the QRS complex to the end of the T wave
QTcF	Corrected QT interval by Fredericia
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
T_{max}	Time of occurrence of C_{max}
$T_{1/2}$	Terminal elimination half-life
VAS	Visual Analogue Scale
WHO	World Health Organization

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 AB (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 AB
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Sweden

Clinical conduct

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Study management

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[redacted]

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Sweden has the lowest prevalence of smoking in Europe, particularly among males. It is widely accepted that one contributory factor to this trend is that snus has replaced cigarettes as the tobacco product of choice among many males and some females.

The nicotine delivery profile of a product is probably one of the main determinants of its efficacy to decrease nicotine craving and, thus, its ability to function as an alternative to cigarettes among current smokers. Oral tobacco, such as snus, is capable of effectively delivering nicotine to the bloodstream through diffusion over the oral mucosa [1]. It may therefore be more satisfactory to smokers than currently available pharmaceutical nicotine replacement therapies (NRTs) with a slower nicotine-delivery profile. Orally administered nicotine, whether in the form of snus or NRT, can never achieve the rapidly rising and very high nicotine levels in the arterial blood to the brain that is typically associated with pulmonary delivery resulting from inhalation of tobacco smoke.

Use of oral tobacco is by definition unassociated with exposure to the many thousands of combustion compounds found in tobacco smoke (many of which are highly carcinogenic and may induce a state of systemic, chronic inflammation), or chronic irritation in the upper and lower airways resulting from the inhalation of tobacco smoke. Therefore, it is generally accepted that use of oral tobacco products has substantially lower health risks than cigarette smoking.

Nicotine is the substance that is the major contributor to the addictive properties of tobacco products, and nicotine exposure may contribute to adverse pregnancy outcomes. In addition, oral tobacco products typically contain low levels of unwanted substances (including nitrosamines and polycyclic hydrocarbons) that have been classified as human carcinogens. So, although the health effects are substantially smaller for oral tobacco compared to cigarette smoking, some adverse effects cannot be ruled out, particularly the effects related to the nicotine exposure.

Traditionally there has been no non-tobacco-based nicotine product intended for recreational use. Despite the vast risk differential between snus and cigarettes in terms of adverse long-term health effects, snus remains a controversial product as it contains tobacco, is intended for recreational use, and is potentially addictive. The tobacco component of snus explains why it contains measurable amounts of unwanted, potentially carcinogenic constituents, albeit at very low concentrations. Oral tobacco-derived nicotine (OTDN) products (*e.g.*, ZYN) have been commercially available for a few years. They have some features that are similar to snus as they come in pouches that are intended to be placed under the upper lip. However, in contrast to snus, these products contain no nitrosamines or polycyclic hydrocarbons, which are the two main classes of unwanted substances in snus. 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 [2] have found that General PSWL (snus) and ZYN Dry release approximately 32-33% and 50-60% of the nicotine during 60 min, respectively, although with large interindividual variation. Extraction is generally larger for small pouches compared to larger pouches, which suggests that surface area, saliva penetration and diffusion factors may be more important determinants of nicotine uptake than pouch weight.

While numerous OTDN products are currently commercially available, only a limited number of these products have, thus far, been scientifically evaluated. It has been suggested that some flavors may enhance nicotine uptake, which has not been fully scientifically investigated for this product category. Similarly, there is a lack of scientific data regarding any possible impact of flavors on pharmacodynamics (PD). Further, the addition of flavors to tobacco products and e-cigarettes have been discussed by regulatory agencies during the last years. The World Health Organization (WHO) seventh report on the scientific basis of tobacco product regulation included a chapter on flavors [3]. Additionally, the United States Food and Drug Administration (FDA) is stressing the need to investigate the effect of flavors on the pharmacokinetics (PK) and PD of nicotine products, as well as a guidance [4] outlining the importance of PD outputs in such investigations.

This study is a part of the effort by Swedish Match to assess if flavoring affects the nicotine uptake, PK and PD of ZYN Dry products. ZYN Dry products have a different product formulation compared to ZYN ULTRA products, for which Swedish Match recently conducted a similar study (study code SM20-02). ZYN ULTRA has a larger pouch dimension and higher moisture, salt, and nicotine content than ZYN Dry which, as mentioned above, may affect nicotine extraction and uptake. While the general PK characteristics of nicotine are known, the nicotine delivery, uptake and subsequent exposure associated with use of ZYN Dry are not. By this approach, the study enables a solid scientific evaluation of the PK and PD properties of ZYN Dry, which will have implications for the assessment of the overall safety profile associated with this emerging product category *vs.* other nicotine products. Given the lack of data on the possible impact of the flavors on the delivery and experience of nicotine in OTDN products, 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. The results of the study will also be made available in the public domain to enhance awareness among these key stakeholders.

The results from this study are intended to be used as a basis in a premarket tobacco product application (PMTA) to the FDA. Prior to introduction and marketing of novel nicotine-based products in the United States, the FDA specifically requires data on nicotine uptake and nicotine effects in order to assess health risks and addiction potential of the new tobacco/nicotine-containing products as compared to existing ones.

6.2 Study rationale

The overarching aim of the study is to evaluate the impact of flavors on nicotine uptake from ZYN Dry products. 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 research subjects to a novel nicotine delivery product with properties that are not yet fully known. However, all research subjects are required to be daily oral tobacco/nicotine pouch users since at least one year (with an average or above-average pouch 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 OTDN products is of pharmaceutical grade, same as the nicotine used in NRT products (*e.g.*, gum, lozenges, mouth spray etc.). Aside from the nicotine, all ingredients used in the OTDN 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 [5-8]. 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 tobacco or OTDN 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.

Each unit of IP (one pouch) will be used for 60 min per study day (see Section 8.1). 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 nicotine. Therefore, acute risks related to the IP administration 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 the nicotine exposure (such as salivation, nausea, and dyspepsia).

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 medical staff at CTC have extensive experience in clinical studies and there are adequate procedures in place to handle unexpected and expected adverse reactions.

Aside from the risks related to the IPs, there may also be risks related to the medical devices used in the study (*e.g.*, indwelling venous catheters). However, these are devices that are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. Study specific evaluations and sampling procedures, like blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable.

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 substantially reduced harm and risk of tobacco-related diseases of the novel OTDN pouches. As the nicotine delivery profile of a product is likely to be central to its acceptability among current nicotine users, it is reasonable to conduct formal clinical studies to assess this feature in more detail.

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 medical examination, which may provide them with information on their general state of 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. OTDN products are an example of such a development, and the use, prevalence and variety of these products has increased globally in recent years. OTDN pouches 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 above, there is a lack of scientific data regarding the potential effects of flavoring on nicotine uptake, PK and PD 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 OTDN products will have less harmful effects on health than tobacco-based nicotine 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 with regard to 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 is a short-term study including 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) [9,10] as well as the Swedish Medical Products Agency (MPA) [11] 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 CTC's policy, which is based on 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 contacted before visits to ensure they do not have any symptoms, and at each visit the subjects' health status is verified before letting them enter the study site.
	Subjects are supplied with hand disinfectant to use during visits to the clinic.
	The study site has elevated the level of cleaning to include regular disinfection of all surfaces in the clinic and clinic kitchen.
Subjects will fall ill with COVID-19 between visits and not be able to show up for IP administration and 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.
Subjects will fall ill with COVID-19-like symptoms during a visit to the study site.	There is a clear action plan if a subject becomes ill during a site visit. The subject is isolated, and if no safety reasons are identified, the subject is sent home and carefully followed up on by site staff. If the subject cannot be sent home because of safety reasons, the subject is isolated and site staff who are in contact with the subject have to wear appropriate personal protective equipment (PPE). The PI will contact the nearest infection clinic and decide on further actions.
The hospital laboratory will not be able to analyze safety samples due to the number of COVID-19 samples.	An accredited laboratory has been contracted as a back-up for the analysis of safety samples.
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.
There will be an increased number of protocol deviations due to the COVID-19 pandemic.	All protocol deviations will be handled according to CTC's standard operating procedures (SOPs).

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective

The primary objective of the study is to evaluate the impact of flavor on nicotine exposure, after the administration of single doses of unflavored ZYN Dry Smooth 6 mg and flavored ZYN Dry Virginia Blend 6 mg.

7.1.1 *Primary endpoint*

PK of nicotine in plasma: The equivalence (90% confidence interval for the ratio between 0.8 and 1.25) in the baseline-adjusted area under the curve from 0 to infinity (AUC_{inf}) based on nicotine plasma concentrations after the administration of single doses of unflavored ZYN Dry Smooth 6 mg and flavored ZYN Dry Virginia Blend 6 mg.

7.2 Secondary objectives

The secondary objectives of the study are:

1. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored and flavored ZYN Dry products.
2. To compare the PK profile between the unflavored and flavored ZYN Dry products.
3. To evaluate the impact of flavors on PD effects, measured as the pulse rate and subjective outcome measures, for all the ZYN Dry products.
4. To evaluate the safety and tolerability of ZYN Dry products in current, daily oral tobacco/nicotine users.

7.2.1 *Secondary endpoints*

The secondary endpoints are:

1. The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored and flavored ZYN Dry products.

2a. PK of nicotine in plasma: The equivalence between the unflavored and flavored ZYN Dry products in the non-adjusted and baseline-adjusted PK parameters maximum observed concentration (C_{max}) and AUC_{inf} based on plasma concentrations of nicotine after the administration of single doses.

2b. PK of nicotine in plasma: The difference between the unflavored and flavored ZYN Dry products in the non-adjusted and baseline-adjusted PK parameters time of occurrence of C_{max} (T_{max}), area under the curve from 0 to 1.5 h ($AUC_{0\text{-}1.5h}$), and AUC from 0 to the last measurable time point ($AUC_{0\text{-}last}$).

3a. The difference between the unflavored and flavored ZYN Dry products for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

3b. The difference between the unflavored and flavored ZYN Dry products for the highest recorded increase (E_{max}) in the subjective parameters “craving” and “satisfaction” measured using a 100 mm visual analogue scale (VAS) after IP administration.

3c. The difference between the unflavored and flavored ZYN Dry products for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 60 min after IP administration.

4. Frequency, intensity, and seriousness of AEs.

7.3 Exploratory objectives

1. To evaluate the impact of flavors on "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice for all the ZYN Dry products.
2. To compare the nicotine extraction-normalized PK parameters AUC_{inf} and C_{max} between the unflavored and flavored ZYN Dry products.

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 "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice between the unflavored and flavored ZYN Dry products, measured on a 3-point scale using a multiple-choice question (MCQ) 60 min after IP administration.
2. The difference in nicotine extraction-normalized AUC_{inf} and C_{max} between the unflavored and flavored ZYN Dry products.

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This is an open-label, randomized, 9-way cross-over, single dose administration study designed to assess the impact of flavors in OTDN pouches on nicotine exposure. The IPs are OTDN pouches, ZYN Dry 6 mg: including 1 unflavored product (ZYN Dry Smooth) and 8 flavored products (ZYN Dry Virginia Blend, Kentucky Bold, Cherry, Dragonberry, Peach, Wine, Bourbon and Dark Rum).

The study will include 42 randomized subjects with the aim to achieve 36 fully evaluable subjects. The subjects are healthy male or female oral tobacco/nicotine pouch users aged ≥21 years who have used oral tobacco/nicotine products for ≥1 year, with a minimum daily consumption of 5 or more pouches. An effort will be made to randomize at least 17 female subjects (~40 %) in the study.

The subjects report to the clinic for a screening visit (Visit 1) followed by 9 treatment visits (Visit 2-10) on separate days. Screening (Visit 1) will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine product use, a brief physical examination, laboratory tests, electrocardiograms (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI, see Table 8.1-1 for details).

Subjects shall abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 h prior to each treatment visit (Visit 2-10). To this end, subjects will be instructed to avoid using such products from approximately 20:00 (8 pm) the day before treatment visits. All IP administrations are performed during the morning hours (08:00 to 12:00) to facilitate abstinence. Subjects should certify abstinence before each treatment starts.

The IPs will be administered as single pouches in a pre-determined randomized order. Subjects will keep the pouch still between the upper lip and gum for 60 min and will be instructed not to manipulate the pouch with the tongue or lips. They will also be instructed not to eat or drink, chew gum, or brush their teeth for 30 min before, during, or 30 min after the IP administration [12].

After 60 min each used pouch will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches from the same batch will serve as references and will be stored at -20 °C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-administration to 6 h after each IP administration. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using VAS questions) at the same pre-defined time points as well as an MCQ 60 min after IP administration.

The total duration for each subject in the study will be approximately 5 weeks, not including the preceding 4-week screening period.

See Table 8.1-2 for a detailed 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	Visit 3-10 Treatment visits
Informed consent	X		
Demographics	X		
Medical/surgical history	X		
History of nicotine use	X		
Inclusion/exclusion criteria	X	X[1]	
Physical examination	X		
Weight and height, BMI	X		
Vital signs (blood pressure and pulse)	X		
Electrocardiogram	X		
HIV, Hepatitis B and C	X		
Pregnancy test[2]	X	X[3]	X[3]
Urine drug screen[4]	X	X	
Alcohol screen[4]	X	X	
Randomization		X	
IP (pouch) administration		X[5]	X[5]
PK blood sampling (plasma)		X[5]	X[5]
Subjective effects (VAS questions and MCQ)		X[5]	X[5]
Pulse rate (pulse oximeter)		X[5]	X[5]
IP (pouch) collection		X[5]	X[5]
Baseline symptoms	X	X	
Adverse events		X	X
Prior and concomitant medications	X	X	X

[1] Confirmation of eligibility criteria.
[2] Only subjects of child-bearing potential.
[3] Only at the discretion of Investigator. Only at the discretion of PI on Visits 2-10.
[4] Additional drug and alcohol tests may be performed at the discretion of the Investigator during treatment visits.
[5] The detailed timing of assessments is outlined in Table 8.1-2.

Table 8.1-2 Detailed schedule of events for each treatment visit (Visit 2-10)

	Visit 2 - 10														
Assessment / Time-point	**Admission**	**-00:15**	**-00:10**	**-00:01**	**00:00**	**00:05**	**00:10**	**00:15**	**00:30**	**00:45**	**01:00**	**01:15**	**01:30**	**02:00**	**04:00**	**06:00**
Inclusion/exclusion criteria	X[1]															
Urine drug screen	X[2]															
Alcohol screen	X[2]															
Pregnancy test	X[3]															
Randomization	X[4]															
IP (pouch) administration					X[5]											
IP (pouch) collection											X[5]					
PK blood sampling (plasma)			X[5, 6]			X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
VAS question (“craving”)			X[5]			X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
VAS question (“satisfaction”)						X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
VAS (“product-liking” and “intent-to-use-again”)											X[5]					
MCQ (“product liking” *vs.* usual product)											X[5]					
Pulse rate (pulse oximeter)			X[5]			X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
Baseline symptoms		X[7]														
Adverse events								X[8]								
Prior and concomitant medications								X								

[1] Only on Visit 2. Confirmation of eligibility before randomization.
[2] Only on Visit 2. Additional drug and alcohol tests may be performed during treatment visits at the discretion of the Investigator.
[3] Only subjects of child-bearing potential. Only at the discretion of PI on Visits 2-10.
[4] Randomization occurs only on Visit 2.
[5] See the timing priority in Section 11.1 and the allowed time deviations in Table 11.1-1.
[6] Pre-administration PK-sample taken at any time within 15 min prior to IP administration, except in direct association with the pre-administration pulse rate assessment at -10 min.
[7] Only on Visit 2. Baseline symptoms experienced prior to first IP administration.
[8] AEs experienced from first IP administration.

8.2 Rationale for study design

This is an open-label, randomized, 9-way cross-over, single dose administration study designed to evaluate the impact of flavors on nicotine uptake from ZYN Dry products.

A cross-over design was chosen to yield a more efficient comparison of treatments (IPs) than a parallel study design, *i.e.*, fewer subjects are required since each subject will serve as its own control. To avoid carryover effects, subjects will abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 h prior to each treatment visit (Visit 2-10).

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

A copy of the randomization list will be generated and provided to the bioanalytical laboratory and to the pharmacokineticist for their analyses.

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 et c.) will be used to reach the target audience. The advertisement 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 screen 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 possible eligibility for the study.

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

If a subject cannot receive the planned dose of IP within 28 days after screening (*i.e.*, the time interval between signing informed consent until dose administration) the subject should be re-screened before proceeding in the study.

9.3 Number of subjects

Forty-two (42) subjects will be included in the study. Approximately 63 subjects will be screened with the aim to achieve 42 randomized subjects and 36 fully evaluable subjects. A fully evaluable subject is defined as a subject who has received all 9 IPs and who has completed all study visits. An effort will be made to randomize at least 17 female subjects (~40 %), however a minimum of 9 randomized female subjects (~20 %) 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 oral tobacco/nicotine products for ≥1 year, with a minimum daily consumption of 5 or more pouches, and who are willing and able to use products with nicotine content ≥1 %
3. Healthy male or female subject aged ≥21 years.

4. Clinically normal medical history, physical findings, vital signs, ECG, and laboratory values at the time of screening, as judged by the Investigator.
5. 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. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
5. 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.
6. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to first IP administration. Positive results that are expected given the subject's medical history and prescribed medications can be disregarded as judged by the Investigator.
7. Current, ongoing use of beta-adrenergic blocking agents (beta blockers), including *pro re nata* (as needed) use.
8. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse, as judged by the Investigator.
9. Plasma donation within 1 month of screening or blood donation (or corresponding blood loss) during the 3 months prior to screening.
10. Subjects who intend to change their nicotine consumption habit, including the intention to stop using nicotine products, within the next 3 months from the screening visit, as judged by the Investigator.
11. 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 to the following restrictions during the entire study duration *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 10).

9.6.1 *General restrictions*

1. Subjects shall abstain from any self-administered oral tobacco/nicotine products as well as smoking (cigarettes and e-cigarettes) for at least 12 h prior to treatment visits and during each treatment visit (Visit 2-10).
2. Subjects shall abstain from eating, drinking, or conducting any other mouth related procedure (*e.g.*, tooth brushing) for 30 min prior to IP administration, during IP use, and for 30 min after IP collection.
3. Subjects shall abstain from any drugs of abuse during the study, *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 10).
4. Subjects shall abstain from alcohol for at least 12 h prior to each treatment visit (Visit 2-10).
5. Subjects must not donate blood or plasma during the study and until 3 months after the last treatment visit (Visit 10).
6. Subjects are not allowed to participate in any other clinical studies during the study period, *i.e.*, from screening (Visit 1) to the last treatment visit (Visit 10).

9.6.2 *Prior and concomitant therapy*

All use of any prescribed medication that includes beta-adrenergic blocking agents (beta blockers), including *pro re nata* use, will be prohibited from admission to the study clinic at Visit 2 until the last study visit (Visit 10). As detailed in exclusion criterion no. 7, subjects currently using beta-adrenergic blocking agents will be excluded from participation in 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.

As detailed in exclusion criterion no. 6, a positive drug screen will exclude a subject from participation in the study. However, positive results that are expected given the subject's medical history and prescribed medications (*e.g.*, opioid analgesics or attention deficit hyperactivity disorder [ADHD] medications) can be disregarded as judged by the Investigator. This does not include positive drug screens resulting from the use of beta-adrenergic blocking agents.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized 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).
- Plasma or blood donation outside of the allowed time windows.

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 to study protocol 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 contact 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 2, the subjects will be randomized to 1 of 6 IP administration sequences. As this is an open-label study, the IP administration sequence to which each subject is allocated for 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, IP administration sequence, period, and IP.

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 AB.

10.1 Identity of test and comparator products

The test and comparator products that will be used in the study are detailed in Table 10.1-1.

Table 10.1-1 Identity of test and comparator products

IP	Nicotine content
ZYN Dry Smooth (unflavored comparator)	6 mg nicotine/pouch
ZYN Dry Virginia Blend	6 mg nicotine/pouch
ZYN Dry Kentucky Bold	6 mg nicotine/pouch
ZYN Dry Cherry	6 mg nicotine/pouch
ZYN Dry Dragonberry	6 mg nicotine/pouch
ZYN Dry Peach	6 mg nicotine/pouch
ZYN Dry Wine	6 mg nicotine/pouch
ZYN Dry Bourbon	6 mg nicotine/pouch
ZYN Dry Dark Rum	6 mg nicotine/pouch

10.2 Manufacturing, packaging, and labelling

All IPs are manufactured and packaged by Swedish Match AB 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 transferred from the original container, weighed, and individually packaged in identical sealed food approved test containers at the Swedish Match analytical lab. The containers will be labelled with unique identification numbers by Swedish Match in accordance with the randomization list.

IPs will be shipped by Swedish Match directly to the research clinic (CTC, Uppsala, Sweden).

10.3 Conditions for storage

IPs will be stored in an access-controlled storage area at CTC in room temperature (20-25 °C).

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, prepared for, and used by each subject, as well as any IPs destroyed 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

A single dose will be given in the morning of each treatment visit (Visit 2-10). Subjects will keep the pouch still between the upper lip and gum for 60 min and are instructed not to manipulate the pouch with the tongue or lips. The subjects will also be instructed not to eat or drink, chew gum, or brush their teeth for 30 min before, during use, or 30 min after the administration of IP [12]. Subjects shall abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 h prior to each treatment visit. To this end, subjects will be instructed to abstain from such products from approximately 20:00 (8 pm) the day before treatment visits (Visit 2-10). All IP administrations will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

10.6 Treatment compliance

Any IP not used or not collected for reference, as well as all empty containers, will be destructed at the site upon confirmation from the Sponsor. The Monitor will perform a final IP accountability reconciliation at the study end to verify that all unused IP is adequately destroyed 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 and Table 8.1-2).

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.

It is important that IP application and collection occur at the scheduled time points in Table 8.1-2. For study assessments, the timing priority order at any time point is:

1. Blood samples for PK
2. Pulse rate assessment
3. VAS questions
4. MCQ

Allowed deviations from actual time are outlined in Table 11.1-1 below.

Table 11.1-1 Allowed deviations from actual time

Time point	IP administration and collection	PK blood sampling (plasma)	Pulse rate (pulse oximeter)	VAS questions	MCQ
-00:15		Pre-administration sample can be taken at any time within 15 min prior to IP administration.[1]			
-00:10			+/- 3 min	+/- 3 min	
-00:01					
00:00	+/- 0 min (IP admin.)				
00:05		+/- 2 min	+/- 2 min	+/- 3 min	
00:10		+/- 2 min	+/- 2 min	+/- 3 min	
00:15		+/- 2 min	+/- 2 min	+/- 3 min	
00:30		+/- 5 min	+/- 5 min	+/- 5 min	
00:45		+/- 5 min	+/- 5 min	+/- 5 min	
01:00	+/- 1 min (IP coll.)	+/- 5 min	+/- 5 min	+/- 5 min	+ 15 min
01:15		+/- 10 min	+/- 10 min	+/- 10 min	
01:30		+/- 10 min	+/- 10 min	+/- 10 min	
02:00		+/- 10 min	+/- 10 min	+/- 10 min	
04:00		+/- 10 min	+/- 10 min	+/- 10 min	
06:00		+/- 10 min	+/- 10 min	+/- 10 min	

[1] Pre-administration PK blood sample should not be taken in direct association with the pre-administration pulse rate assessment at -10 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*

History of oral tobacco/nicotine products use in terms of brands, average consumption per day during the last 30 days, and duration of use (years), history of smoking in terms of number of cigarettes per day during the last 30 days, as well as history of vaping in terms of number of days during last 30 days, will be obtained by subject interview.

11.2.5 *Eligibility criteria*

Eligibility criteria should be checked during screening and verified before randomization and administration of first IP on Visit 2. The criteria are specified in Sections 9.4 and 9.5.

11.2.6 *Physical examination*

A brief physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

11.2.7 *Weight and height, BMI*

Weight and height will be measured without shoes. BMI will be calculated to 1 decimal point from the recorded height and weight.

11.2.8 *Vital signs*

Systolic and diastolic blood pressure and pulse will be measured in supine position after 10 min of rest. Any post-IP measurements of pulse rate (using pulse oximeter) judged as "abnormal, clinically significant" by the Investigator will be reported as AEs.

11.2.9 *ECG*

Single 12-lead ECG will be recorded in supine position after 10 min of rest using an ECG machine. Heart rate (HR) and PR, QRS, QT, and QTcF intervals will be recorded.

ECGs will be reviewed and interpreted on-site by the Investigator.

11.2.10 *HIV and hepatitis B and C*

Subjects will be tested for HIV and hepatitis B and C prior to inclusion into the study. Any positive results will exclude the subject from participating in the study.

11.2.11 *Pregnancy test*

All female subjects of child-bearing potential will do a urine pregnancy test at screening (Visit 1) as well as at the discretion of the Investigator during treatment visits (Visit 2-10).

11.2.12 *Drug and alcohol tests*

Subjects will be screened for drugs of abuse (urinalysis) and alcohol (breath test) at screening (Visit 1) and the first treatment visit (Visit 2). Additional drug and alcohol tests may be performed during remaining treatment visits (Visit 3-10) at the discretion of the Investigator.

11.2.13 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until the first IP administration (*i.e.*, an event that occurs during the screening period). Such events are not AEs and will be recorded as baseline symptoms in the Medical History Log in the eCRF.

11.2.14 *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 first IP administration, prior to IP administration, and as concomitant if ongoing on the day of the first IP administration, started or stopped after the first IP administration. To distinguish between prior and concomitant medications on the first treatment 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 eCRF.

Any use of concomitant medication from screening until the last treatment visit (Visit 10) 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 Assessments related to primary endpoints

11.3.1 *Nicotine plasma concentration and pharmacokinetic sampling*

Venous blood samples (approximately 3 mL) for the determination of plasma concentrations of nicotine after IP administration will be collected through an indwelling venous catheter at pre-defined time-points; pre-administration (within 15 min prior to dose), and 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 1 h:15 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration, see Table 8.1-2. It is important that blood PK sampling does not deviate from the planned time more than the allowed time deviations outlined in Table 11.1-1.

The date and time of collection of each sample will be recorded in the eCRF.

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged to separate plasma. The separated plasma from each blood sample will be divided into 2 aliquots in pre-labelled cryotubes and frozen at -20°C.

For further details see the lab manual.

Plasma samples for determination of plasma concentrations of nicotine will be analyzed by Lablytica by means of a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

11.4 Assessments related to secondary endpoints

11.4.1 *Pharmacokinetic sampling related to secondary endpoints*

PK sampling related to secondary endpoints 2a and 2b is part of the procedure described in Section 11.3.1 above.

11.4.2 *Pharmacodynamic effects of ZYN Dry*

The PD effects of the IPs will be assessed by measuring pulse rate and subjective parameters (using VAS and MCQ) at pre-defined time points as described below and in Table 8.1-2.

11.4.2.1 Pulse rate

The pulse rate will be monitored using a pulse-oximeter and will be spot-assessed at -10 min pre-administration, as well as at 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 1 h:15 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration (see Table 8.1-2).

Allowed deviations from actual time are outlined in Table 11.1-1.

11.4.2.2 Subjective parameters

Subjective parameters ("craving", "satisfaction", "product-liking" and "intent to use again") will be rated using VAS, anchored with "not at all" to "extremely", or "very likely" for the "intent to use again" parameter.

The "craving" parameter will be assessed with the question "Right now, how strong is your urge to snus?" at the same pre-defined time points as the pulse-rate assessments: -10 min prior to IP administration, and 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 1 h:15 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration.

The "satisfaction" parameter will be assessed with the question "Right now, is the product satisfying?" at the pre-defined time points: 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 1 h:15 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration.

In addition, "product-liking" and "intent to use again" parameters will be assessed at 60 min post-administration with the questions "How much did you like the product?" and "How likely are you to use this product again in the future?", respectively.

Time points for each of the VAS questions are outlined in Table 8.1-2, and allowed deviations from actual time are outlined in Table 11.1-1.

11.4.3 *Nicotine extraction from pouches*

Used pouches will be collected after 60 min (+/- 1 min) of use for the determination of residual nicotine in the IPs.

All the collected pouches will be frozen immediately at -20°C. Pouches for extraction of nicotine will be analyzed by Swedish Match.

11.5 Assessments related to exploratory endpoints

11.5.1 *Exploratory subjective parameter*

The subjective parameter "product-liking" *vs.* the subject's usual snus/nicotine product of choice will be assessed at 60 min with the MCQ "How much did you like the product compared with your usual snus or nicotine product of choice?" on a 3-point scale: "1 = To a lesser extent, 2 = To the same extent, 3 = To a greater extent."

The allowed deviation from actual time for the MCQ is outlined in Table 11.1-1.

11.6 Adverse events

The PI is responsible for ensuring that all medical staff involved in the study is familiar with the content of this section and the content of the CTC's SOPs regarding emergencies.

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 IP administration (Visit 2) until the last treatment visit (Visit 10).

Any AE with start date on the day of IP administration must be recorded with start time.

At Visit 10, 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 [13]. 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 relationship*

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 paper 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 10), 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 possible 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*

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

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

An overdose should be documented as follows:

- An overdose with associated AE is recorded as the AE diagnosis/symptoms in the AE Log of the eCRF.
- An overdose without associated symptoms is only reported in the subject's medical records.

11.7 Appropriateness of measurements

All methods used for safety assessments are commonly used in standard medical care and in Phase I clinical studies. Non-compartmental analysis (NCA) of PK parameters is standard for Phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.3.1.

12.2 Volume of blood

The anticipated total volume of blood collected during the whole study will be approximately 330 mL from each subject. For reference, a regular blood donation consists of between 350 mL to 450 mL (±10 %) for persons weighing at least 45-50 kg [14].

12.3 Handling, storage, and destruction of laboratory samples

The plasma samples for the analysis of PK parameters will be registered in CTC's biobank (Swedish Health and Social Care Inspectorate biobank registry number 893). The samples will be stored at <-20 °C until analyzed and disposed of after the CSR has been finalized.

Any remains from the safety laboratory samples will be disposed of after analyses.

12.4 Chain of custody of biological samples

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

CTC 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.

12.5 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 maintain 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 [15] and are consistent with the ICH E6(R2) guideline for GCP [16], the EU Clinical Trials Directive [17], 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 possible 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 [18], 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 representatives 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 [18] 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 are 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 AB 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 AB´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. CTC has a company insurance covering services performed by CTC.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before enrolment of the first study subject, a Sponsor representative or delegate will perform a study initiation visit at the research clinic. The requirements of the CSP and related documents will be reviewed and discussed, and the investigational staff will be trained in any study specific procedures and system(s) utilized.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study 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 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 Q1 2022 and to be completed by Q2 2022.

A subject is considered to have completed the study if he/she has completed all treatment visits in the study (Visit 2-10). Each subject who completes the study will participate in the study for a period of approximately 5 weeks, not including the preceding screening period of approximately 4 weeks.

The end of the study is defined as the last visit, *i.e.*, the last treatment visit (Visit 10), 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 CTC. The report 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 at bedside (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 are not recorded in the eCRF. Data may be received in electronic format. Key variables are defined in order 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 have 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 with descriptive statistics in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value, Q1-Q3 (interquartile range [IQR]). In addition, for the parameters AUC and C_{max} the geometric mean and coefficient of variation (CV) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by IP, and by assessment time. Individual subject data will be listed by subject number, IP, 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 collection time point prior to each IP administration.

No adjustment for multiple comparisons will be performed. All formal comparisons will be made towards a designated reference product and all significant findings will be reviewed for medical relevance.

No imputation of missing data will be performed.

17.2 Determination of sample size

Previous studies [6,7] have shown that the *in vivo* extracted fraction of nicotine has a CV ranging between 31-33%, and similar CVs for AUC_{inf}. Assuming no difference between products, defining equivalence as a 90% CI of least squares means (LSMeans) entirely in the range 0.8-1.25, and using a CV of 32.5%, a power of 80% and a significance level of 10 %, 36 evaluable subjects will be needed. To account for a dropout rate of 15%, 42 subjects will be randomized.

17.3 Pharmacokinetic analysis

The PK analysis will be based on the pharmacokinetic analysis set (PKAS) and performed by CTC. The PK parameters will be calculated by NCA using the software Phoenix WinNonlin® version 8.1 or later (Certara Inc, Princeton, New Jersey, U.S.A.). In addition to AUC_{inf} and terminal elimination half-life ($T_{1/2}$) parameters, other non-compartmental PK parameters will be determined in relation to secondary endpoints (see Section 17.7.2 below). Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

For AUC_{inf}, the area under the plasma concentration *vs.* time curve will be calculated to the time point of the last quantifiable plasma concentration of nicotine and then extrapolated to infinity using the concentration in the last quantifiable sample and the estimated terminal elimination rate constant ($Lambda_z$).

PK data will be presented for each IP using summary statistics. This data will be presented in terms of N, arithmetic mean, SD, minimum and maximum value. For AUC and C_{max}

parameters, the geometric mean and CV will be presented. Categorical data will be presented as counts and percentages as applicable.

17.4 Analysis data sets

17.4.1 *Full analysis set*

The Full Analysis Set (FAS) will consist of all subjects who have been randomized and received at least 1 dose of IP and who have at least 1 post-baseline data point. This population will be used as the safety analysis set.

17.4.2 *PK analysis set*

The PK analysis set (PKAS) will consist of all subjects who received at least 1 IP administration and provided at least 1 evaluable PK-profile and no major deviation judged to compromise the PK analysis. Individual PK values and/or full profiles for explicit IPs may be excluded from the analysis as specified in the SAP.

17.4.3 *Additional analysis datasets*

Additional datasets may be created as needed for exploratory analyses.

17.5 Description of study population

17.5.1 *Demographics and baseline characteristics*

Demographics, weight, height, BMI as well as history of oral tobacco/nicotine use, and smoking will be presented by descriptive statistics. All data will be listed by subject.

17.5.2 *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.

17.5.3 *Treatment compliance*

The number of subjects treated with each IP will be presented through descriptive statistics and listings.

17.5.4 *Physical examination, ECG, and vital signs*

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.

17.6 Analysis of primary endpoint

17.6.1 *Analysis of equivalence of ZYN Dry Smooth and ZYN Dry Virginia Blend (AUC_{inf})*

The primary endpoint is the equivalence in AUC_{inf} after the administration of single doses of unflavored ZYN Dry Smooth 6 mg *vs.* the flavored ZYN Dry Virginia Blend 6 mg.

AUC_{inf} will be calculated as described in Section 17.3 above.

Equivalence will be assessed using a mixed model with the natural log of AUC_{inf} as the dependent variable, treatment as a fixed effect and subject as a random effect. The Kenward-Rogers approximation for degrees of freedom will be used. The estimated LSMeans difference between ZYN Dry Smooth and ZYN Dry Virginia Blend will be back-transformed into the original scale to present the ratio of geometric LSMeans as well as the corresponding 90 % confidence interval. If the 90 % confidence interval of this ratio falls within the range 0.8-1.25, the IPs will be considered equivalent. The model will be estimated for both non-adjusted and baseline-adjusted AUC_{inf}.

17.7 Analysis of secondary endpoints

17.7.1 *In vivo extracted amount and fraction of nicotine*

The difference between the nicotine content of an unused reference pouch and the used study pouch will be used to calculate the *in vivo* extracted amount of nicotine for each IP. The mean of the extracted amount (mg/unit) and extraction fraction (%) of nicotine for each IP pouch will be calculated. The amount of nicotine in the reference pouches and in used pouches will be presented through descriptive statistics.

17.7.2 *Pharmacokinetics of nicotine in plasma: Unflavored ZYN Dry Smooth vs. flavored ZYN Dry products*

The following non-compartmental PK parameters will be determined for each IP: AUC_{inf}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}1.5h}$, C_{max}, T_{max} and $T_{1/2}$. Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

C_{max} and T_{max} will be derived from the observed nicotine plasma concentration data. $AUC_{0\text{-}1.5h}$ and $AUC_{0\text{-}last}$ will be calculated using log-linear trapezoidal interpolation. Calculations will be based on the actual sampling times recorded during the study. Concentrations below the lower limit of quantification (LLOQ) occurring before C_{max} will be treated as zero. Concentrations below LLOQ occurring after C_{max} will be omitted from the analysis. All baseline-adjusted PK parameters will be corrected for nicotine plasma concentrations at baseline (pre-administration).

AUC_{inf} will be calculated as described in Section 17.3 above.

The equivalence in AUC_{inf} and C_{max} between the unflavored ZYN Dry Smooth (comparator product) and each flavored ZYN Dry product will be estimated as for the primary endpoint, described in Section 17.6.1. Equivalence will be estimated for both non-adjusted and baseline-adjusted AUC_{inf} and C_{max}.

17.7.3 *Pharmacodynamic effects of ZYN Dry*

The analyses of pulse rate as a measure of the PD effect of nicotine will be further described in the SAP. Data will be presented as summary tables of descriptive statistics and plots of pulse rates over time for each IP.

The subjective parameters "craving", "satisfaction", "product-liking" and "intent to use again", measured by VAS, will be summarized for each IP using descriptive statistics as a total score during the IP administration phase. The relative and absolute change from baseline will also be calculated for the subjective parameter "craving" for each IP.

To determine the maximum PD effect attributable to the IP, the E_{max} as well as time to first instance of E_{max} will be calculated for the pulse rates as well as for the subjective parameter "craving". For the subjective parameter "satisfaction", which does not have a baseline, the maximum value will be recorded as E_{max} along with time to E_{max}.

17.7.4 *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.

17.8 Analysis of exploratory endpoints

17.8.1 *Exploratory subjective parameter*

The subjective parameter "product-liking" *vs.* the subjects' usual snus/nicotine product of choice, measured through MCQ, will be summarized for each IP using descriptive statistics.

17.8.2 *Extraction-normalized AUC_{inf} and C_{max}*

Nicotine extraction-normalized PK parameters AUC_{inf} and C_{max} (with and without baseline-adjustment) will be determined for each IP. Extraction-normalized AUC_{inf} and C_{max} data will be presented for each IP using summary statistics.

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19 SIGNATURES

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