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Serbian Smoking Reduction/Cessation Trial (2SRT)

Clinical Study Protocol SM 07-01

SIGNATURE PAGE

Sponsor Approval:
Signature: Date:

Name:

Investigator Agreement: I have read the protocol and agree to conduct the study as outlined herein.

Signature: Date:

Name:

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SYNOPSIS

TITLE OF STUDY	**Serbian Smoking Reduction/Cessation Trial (2SRT)**
INVESTIGATORS & STUDY CENTERS	Professor Robert Nilsson, Stockholm, International Coordinating Investigator Dr Ruza Antic, Belgrade, Principal National Investigator Participants will be recruited at four centers in the city of Belgrade, Serbia
PHASE OF DEVELOPMENT	**Phase III**
OBJECTIVES	**To assess the efficacy of a traditional Swedish smokeless tobacco product ("snus") to reduce smoking among adult smokers in Serbia**
DESIGN	**Randomized, placebo-controlled, double-blind trial**
PLANNED SAMPLE SIZE	**500**
DIAGNOSIS AND KEY SUBJECT SELECTION CRITERIA	**Males and females in good general health aged 20-65 years regularly smoking >10 cigarettes per day for more than 1 year, who are motivated to reduce or quit smoking**
TREATMENTS	**1. Traditional, low-nitrosamine Swedish snus in 0.5 or 1.0 g sachets ad libitum** **2. Placebo snus (without tobacco or nicotine)**
MAIN PARAMETERS OF EFFICACY	**Primary end-point:** • "Smoking reduction" at 24 weeks, defined as a self-reported reduction of $\geq$50% compared to baseline in the average number of smoked cigarettes per day during week 20-24, verified by a reduced concentration of carbon monoxide (CO) in exhaled air of at least 1 ppm **Secondary end-points:** • "Smoking reduction" at 12 weeks • Smoking cessation at 12 and 24 weeks defined as self-reported total abstention from cigarettes during the preceding 4-week period verified by a concentration of CO in exhaled air of <10 ppm • Smoking cessation at 36 and 48 weeks among those who achieved smoking reduction at 24 weeks (cessation here defined as self-reported total abstention from cigarettes during the preceding 4, 12, or 24-week period, verified by a CO-concentration in exhaled air of <10 ppm • Clinical tests and biomarkers at 12 and 24 weeks among all participants, and at 36 and 48 weeks among those who achieved smoking reduction at 24 weeks, including body weight, blood pressure, CO in exhaled air, measures of lung function ,total S-WBC, S-CRP, total S-cholesterol, S-HDL, S-LDL, S-fibrinogen, S-cotinine

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
BMI	body mass index
CRF	case report form
CRP	C-reactive protein
CRO	contract research organization
GCP	good clinical practice
GothiaTek®	Industrial standard under which snus products are manufactured
EU	European Union
FEV%	$FEV_{1.0}$ /FVC
$FEV_{1.0}$	forced expiratory lung volume during the first second
FVC	forced vital capacity
HDL	high-density lipoprotein
IEC	independent research ethics committee
IRB	institutional review board
ITT	intent-to-treat
LDL	low-density lipoprotein
NCS	not clinically significant
NRT	nicotine replacement therapy
ppm	parts per million
TSNA	tobacco specific nitrosamines
WBC	white blood cell count
WHO	World Health Organization
WHOART	World Health Organization Adverse Reaction Terms

1. INTRODUCTION

In developed countries (e.g. Europe, North America, Japan, Australia, and New Zeeland) tobacco-related disease is the single most important avoidable cause of premature death among males. In these countries an average of about 25% percent of male deaths has been attributed to tobacco smoking (Peto et al, 2003). It follows that policies to reduce smoking-related disease would have a considerable potential for reducing future morbidity and mortality. In this context it is of special importance to assess country-specific data for Serbia in comparison with other European countries, where Serbia shares with Croatia top positions for male all-cause mortality attributable to smoking (Peto et al., 2003).

Sweden, on the other hand, demonstrates a unique pattern in terms of smoking-related disease. Not only are male smoking-related deaths radically fewer than in other European countries, but Sweden is also the only country where males have a lower overall risk of smoking-related death than women (IARC, 1997). This finding can be explained by the fact that during the last decades male smoking has decreased to a considerably larger extent in men than in women, largely due to the prevalent use in men of "snus", a traditional Swedish oral tobacco product, as a smoking cessation aid or replacement for cigarettes (Henningfield & Fagerström, 2001). The use of low-nitrosamine smokeless tobacco of the Swedish type is associated with health risks that are only a fraction of those caused by smoking (Kozlowski, L.T, 2002; Foulds, Ramström, Burke, Fagerström. 2003; Levy et al., 2004).

Other types of smokeless tobacco products extensively used in India and parts of Africa have been associated with substantial risks of both oral cancer and cardiovascular disease (IARC, 1985, Idris et al, 1994). However, these products are distinctly different from Swedish snus in that they typically contain much higher levels of TSNA:s, and are often combined with betel and areca nuts which contain non-tobacco substances that are highly carcinogenic.

The manufacture of Swedish snus is regulated according to Swedish laws for food-stuffs and according to an industrial standard (GothiaTek®) that includes limits for tobacco-specific substances, such as TSNA:s, as well as for potentially toxic substances present in all food-stuffs, such as, heavy metals, pesticide residues, and mycotoxins (www.gothiatek.com). In addition, Swedish snus is traditionally manufactured using a heating process similar to pasteurization which renders the product virtually sterile, which helps to ensure product stability during storage.

During the last years, the public health authorities in Serbia have initiated antismoking campaigns and bans on smoking in public places. However, due to the difficult financial situation after the Balkan wars, the funding for such campaigns have been limited, as well as for modern pharmaceutical smoking cessation products. An alternative nicotine delivery device providing aid in smoking cessation is lacking that is both acceptable from the point of efficacy and cost.

In summary, in Serbia a reduction of the smoking-related burden of disease is an important public health issue. The experience from Sweden and other countries indicates that substantial risk reduction due to smoking related disease - tobacco harm reduction - is achievable, despite limited financial resources.

Main attempts to reduce smoking related disease are directed towards prevention of onset of smoking in young people, and cessation/reduction of smoking among adult, current smokers. Obviously, both approaches are important, but differ with respect to their short-term versus long-

term impact. Most of the smoking-related deaths for the next 50 years will occur among existing smokers as cancer, cardiovascular disease and lung disease related to smoking typically occur after several decades of smoking. Consequently, prevention of onset of smoking among young people will not have a major influence on mortality until today's teenagers reach the age where smoking-related disease becomes a significant cause of death, i.e. towards the mid of this century. On the other hand, cessation of smoking starts influencing death risk already after a few years (Peto et al, 2003).

Many smokers, in particular heavy smokers, have great problems quitting smoking, and the use of alternative nicotine delivering devices may be of considerable assistance to promote abstinence as evidenced by numerous controlled clinical trials of the role of NRT:s in smoking cessation (George & O'Malley, 2004). However, the low success rate for alternative nicotine delivering devices such as nicotine chewing gum, nicotine patch and nicotine spray are well documented. This observation seems to be due to the fact that the kinetics of nicotine absorption differs greatly from that associated with smoking (Fant et al., 1999). Also, the cost of these nicotine sources is high. More recently, other pharmaceutical smoking cessation aids have been introduced, such as, bupropion and vareniclide (Schnoll & Lerman, 2006). Although the long-term success rates associated with these new therapies appear marginally better than with NRT, the drug costs are prohibitively high for a Serbian public health setting. In addition, due to the potential side effects of these new therapies including neuropsychological problems and nausea, they probably remain prescription drugs with a limited impact on smoking cessation from a public health perspective.

Oral snuff (smokeless tobacco), for which the kinetics of nicotine delivery is more similar to that of smoking compared to NRT, offers another possibility for nicotine replacement therapy. The Swedish experience provides strong indirect support to the notion that snus with low levels of nitrosamines, provides an effective aid in smoking cessation (Bates et al., 2003). According to the Swedish National Board of Health (2005) subjects who used snuff for the purpose of smoking cessation had a 50% higher probability of remaining non-smokers than those who quit without any alternative nicotine source. The cost for one days´ consumption of Swedish low-nitrosamine snus is not higher than for one package of cigarettes.

Clinical experience on smoking cessation from the Nicotine Institute in Vienna (Kunze, personal communication) indicates that recruitment to smoking cessation programs is more successful if the goal proposed to the subjects entering a smoking cessation program is focused on reducing smoking rather than emphasizing total cessation. Many smokers have previously made unsuccessful attempts to stop smoking, and therefore might abstain from participating in a program if the requirement is total abstention. However, for several subjects treatment with a cigarette substitute containing nicotine will reduce smoking drastically, and eventually to such a low level that smoking cessation is facilitated. The current trial will, therefore, adopt this general philosophy.

Considerations as those presented above constitute the rationale for conducting a controlled clinical trial in Serbia among adult smokers with the goal to reduce smoking, and eventually stop smoking, using low-nitrosamine snus manufactured according to the Swedish GothiaTek® standard.

Some types of oral smokeless tobacco have been associated with the induction of cancers in the head/neck region (Winn et al, 1986, IARC, 1985; Idris et al., 1994). Consequently, the EU tobacco directive previously required that packages of snuff should carry a cancer warning.

However, the European Commission decided that the cancer warning be dropped in 2001, because large, Swedish epidemiological studies showed no increase of cancer risk associated with the use of Swedish snus (Lewin et al., 1998; Schildt et al., 1998).

A recent extensive public health report from the Swedish National Board of Health (2006) emphasized that:

> "Although we cannot exclude health risks associated with snus…the conclusion is that these are very small in comparison with smoking".

This conclusion has been supported by prestigious institutions like the Royal College of Physicians in Great Britain (2002) as well as by the Institute of Medicine of the National Academies of Sciences, Washington, D.C. (IOM, 2001).

The use of oral smokeless tobacco is virtually unknown in Serbia. A limited feasibility study was therefore conducted in Belgrade during 2005 where 21 smokers were offered different Swedish snus products, and 18 were offered the Nicorette® nicotine patch. The main objectives for this study were to assess the acceptability of various snus products in a Serbian setting, as well as to assess possible potential problems that could be expected in a full scale study. Out of the 21 participants who tried snus, a total of ten preferred an eucalyptus flavored brand, seven an liquorice flavored brand, four a menthol brand, and none liked the taste of a regular unflavored Swedish snus brand.

One main obstacle in conducting the feasibility study was poor motivation among some male participants, perhaps due to a low awareness of the health risks associated with smoking. For the whole group of smokers, however, there was a marked reduction of average CO levels in exhaled air at the end of the one month period indicating a substantial reduction of the number of cigarettes smoked. The study also clearly demonstrated that, if properly flavoured, oral moist tobacco like Swedish snus was acceptable to both Serbian male and female smokers.

This protocol has been developed in collaboration with an international advisory panel consisting of:

- Prof. Michael Kuntze, University of Vienna, Austria
- Dr. Karl-Olof Fagerström, Fagerström Consulting, Hälsingborg, Sweden
- Prof. Robert Nilsson, University of Stockholm, Sweden

Prof. Nilsson will function as International Coordinating Investigator in the trial, and Dr Ruza Antic, Belgrade, Serbia will be the National Principal Investigator.

2. STUDY OBJECTIVES

The primary objective of this study is to assess the efficacy of a traditional Swedish low nitrosamine smokeless tobacco product ("snus") to help adult cigarette smokers in Serbia to substantially reduce their smoking or completely stop smoking.

The secondary objective of this study is to assess the effects of the intervention on clinical tests and biomarkers related to lung function, risk of cardiovascular disease, and other health outcomes.

3. INVESTIGATIONAL PLAN

3.1 Description of Overall Study Design and Plan

This multi-center, double-blind, placebo-controlled, randomized, clinical trial is designed to evaluate snus versus placebo as an aid to reduce smoking among adult cigarette smokers in Serbia.

The snus and the placebo product come in sachets (or pouches) that are to be placed in the mouth between the upper gingiva and cheek. The sachets are delivered in plastic containers each with 24 sachets. The participants can choose from two different sachet sizes (0.5 and 1g) and two different flavors (liquorice and eucalyptus). The rationale for using different sachet sizes is that many individuals feel that the standard 1g sachet is too big to be easily retained in the mouth. On the other hand, the smaller sachet contains less tobacco and may not deliver enough nicotine to alleviate nicotine abstinence in all smokers. The selected flavors were those favored by most participants in the mentioned feasibility study.

The contents of snus and the placebo product are described in detail in an appendix to this protocol. The content of both products complies with the industrial standard GothiaTek®.

It should be noted that the product used as placebo in this trial is a snus replacement product that does not contain nicotine, but is widely marketed in Sweden under the brand name “Onico”.

The participants will be instructed to try to refrain from cigarettes through the use of their allocated study product when they feel an urge to smoke. The sachet should be retained in the mouth during at least 20-30 minutes (at which time the blood nicotine levels reach a maximum). If the participants still feel an urge to smoke after 30 minutes, they can do so. The participants will be encouraged to substitute as many cigarettes as possible with the study products, although total abstention from cigarettes is not a primary goal during the first 24 weeks. Those who are able to achieve a substantial reduction of their self-reported cigarette consumption at 24 weeks (verified by a decreased level of CO in exhaled air) will be encouraged to quit smoking during the ensuing 24-48 week follow-up. Those who cannot achieve a substantial reduction at 24 weeks are defined as treatment failures and will not be followed beyond 24 weeks.

The amount of nicotine needed to prevent withdrawal symptoms among smokers varies considerably. The amount of study products used by the participants is therefore also expected to vary and is dependent on the extent to which the products actually can replace cigarettes. The participants will therefore be instructed to use the products ad libitum. They will be informed that one 1 g snus sachet typically can replace one cigarette. There is a possibility of nicotine overdosage, particularly among those who both use cigarettes and snus since the participants, at least in the early phase of the trial period are unfamiliar with the effects of snus. However, such overdosage is typically self-limiting since it will result in symptoms that are familiar to most habitual smokers, that is, nausea, tachycardia, cold sweat etc. The symptoms are quickly reversible upon cessation of smoking or use of snus.

3.2 Selection of Study Population: Information Seminar & Baseline Visit

Potential participants will be invited (through posters and ads, see appendix) to an information seminar with c. 10-20 attendees where the goals of the study as well as the means of its achievement are explained. Information is provided about health risks associated with smoking, as well as about possible alternative aids available for smoking cessation. The physiological

effects of nicotine will be outlined, and an account given of the Swedish experience with snus including potential health risks associated with different types of smokeless tobacco products. The trial inclusion and exclusion criteria will be mentioned.

A few days-weeks after the seminar, potential trial participants are invited to a baseline visit during which written informed consent to participate will be obtained. Other activities at the baseline visit are described in detail in section 3.4 Assessments.

Participants are healthy, adult cigarette smokers aged 20-65 years who habitually smoke >10 cigarettes per day, who have smoked daily for >1 year, and who are motivated to substantially reduce or completely stop smoking but who are unable to do so without help.

Specific entry criteria are detailed in Sections 3.2.1 and 3.2.2.

3.2.1 Inclusion Criteria

Subjects meeting all of the following inclusion criteria can be considered for admission to the study.

1. The subject is aged between 20 through 65 years
2. The subject smokes >10 cigarettes per day (average daily consumption during past month)
3. The subject has smoked daily for >1 year
4. The subject is motivated to substantially reduce or quit smoking
5. The subject is in good general health
6. The subject accepts not to take NRT or any other non-protocol treatment to facilitate smoking cessation during the study period

3.2.2 Exclusion Criteria

Subjects meeting any of the following exclusion criteria will not be enrolled in the study.

1. The subject has uncontrolled hypertension (systolic >140 mg Hg, diastolic >90 mg Hg)
2. The subject has a history of coronary heart disease or other significant heart condition
3. The subject has a history of another significant medical condition that may interfere with study procedures
4. The subject is a pregnant or nursing mother
5. The subject currently abuses alcohol or drugs
6. The subject has current active oral disease that may interfere with use of snus
7. The subject has significant psychiatric or psychosocial problems that may interfere with study procedures
8. The subject has used any type of pharmaceutical or other products for smoking reduction or cessation within the past 3 months

3.2.3 Removal of Subjects from Therapy or Assessment

A subject will be considered to have completed the study when he or she completes the final assessment visit at week 48. If a subject is discontinued at any time after entering the study, the investigator will make every effort to contact the subject and complete the Premature discontinuation form as shown in *Section 3.4.1, Schedule of Assessments*.

A termination case report form (CRF) page should be completed for every randomized subject, whether or not the subject completed the study. The reason for any early discontinuation should be

indicated on this form. The primary reason for a subject withdrawing prematurely should be selected from the following standard categories of early termination:

- *Failure to achieve "smoking reduction" at 24 weeks (>50% reduction of self-reported number of smoked cigarettes during week 20-24, and CO in exhaled air <1 ppm, compared to baseline):* such subjects are unlikely to stop smoking during subsequent follow-up which is the aim of the study after 24 weeks.
- *Protocol Violation*: The subject's findings or conduct failed to meet the protocol entry criteria or failed to adhere to the protocol requirements (e.g., treatment noncompliance, failure to return for defined number of visits). The violation necessitated premature termination from the study. Clinical visits are scheduled with a -1/+1 week window. Visits during this two-week window are not considered to be protocol violations. Every effort should be made to establish contact with participants who fail to show up for scheduled visits to determine the cause of the non-compliance. Failure to complete one scheduled visit within the mentioned -1/+1 week window due to disease or other factors outside the participant's control is acceptable whereas failure to complete two consecutive visits shall be regarded as a protocol violation that necessitates premature study termination.
- *Lost to Follow-Up*: The subject stopped coming for visits and study personnel were unable to contact the subject
- *Withdrawal of consent*: The subject desired to withdraw from further participation in the study in the absence of an investigator-determined medical need to withdraw. If the subject gave a reason for withdrawing, it should be recorded in the CRF.
- *Adverse Event (Adverse Reaction)*: Clinical events occurred that in the medical judgment of the investigator for the best interest of the subject are grounds for discontinuation. This includes serious and nonserious adverse events regardless of relation to study medication.
- *Death:* The subject died.
- *Other*: The subject was terminated for a reason other than those listed above

3.3 Treatments

3.3.1 Details of Study Treatments

Comprehensive information about the study products are given in an appendix to this protocol. All products used as part of the trial will be provided free of charge to the participants by the sponsor, and will be distributed by the central trial office. For participants who wish to continue with snus after completion of the study, the sponsor intends to make snus available from commercial retailers within the city of Belgrade, unless this is made impossible by regulatory or other circumstances outside of the sponsor's control.

3.3.2 Dosage Schedule

Use of snus (or placebo) within the trial is ad libitum. When participants feel an urge to smoke, they are instructed to try their allocated study product for at least 20-30 minutes. If they still feel an urge to smoke, they can do so. Participants will be informed that one 1 g snus sachet typically can replace one cigarette. They will also be informed that nicotine overdosage may occur with excessive use of the product, particularly with concomitant smoking, but those symptoms (typically nausea, tachycardia, etc) quickly subside upon cessation of smoking or use of the product. The participants will be instructed to replace as many cigarettes as possible with their allocated study product. Those who achieve "smoking reduction" at 24 weeks will be encouraged to quit smoking totally. The average snus consumption among snus users in Sweden who do not smoke is about 10-15 sachets per day.

3.3.3 Treatment Assignment

After providing informed consent at the baseline visit, the subjects will be randomly allocated to either active snus or placebo snus. Stratification will be made according to treatment centre. Randomization will be done by telephone to a central office where participant's identifiers are recorded. The random allocation will be done according to a computer-based algorithm.

3.3.4 Product Packaging and Blinding

As this is a double-blind trial, the study products will be distributed from the central office and will be labeled with the participant's identifiers. Only the central office will have access to the key linking each participant to their allocated treatment.

3.3.5 Treatment Compliance

Self-reported number of cigarettes smoked daily and snus sachets used per day during the past week will be recorded each week. A simple participant diary will be used; a sample diary is enclosed as an appendix.

3.3.6 Prior and Concomitant Illnesses and Treatments

Prior and Concomitant Illnesses

As this study is to be performed in healthy subjects, there should be no significant concomitant illnesses at the time of entry into the study. Illnesses first occurring or detected during the study, or a significant deterioration of a pre-existing condition will be documented as adverse events in the CRF. All data on medical history etc. collected as part of this trial will be based on the participant's self-reported information.

Prior and Concomitant Treatments

Any concomitant medication is acceptable except NRT or other treatments used for smoking cessation, for instance, bupropion or vareniclide. If a subject has used such treatments previously, they must have been stopped more than three months ago.

3.4 Assessments

3.4.1 Schedule of Assessments

The procedures to be performed throughout the study are outlined in the flow chart of scheduled events which is enclosed as an appendix to this protocol. A detailed description of each assessment may be found in Section 3.4.2, Description of Assessments.

3.4.2 Description of Assessments

Unless otherwise indicated, all assessments listed below will be performed by the investigator or other regular study personnel. If the subject terminates the study or drops out before week 48, the premature discontinuation form in the CRF should be filled in.

Clinical visit at baseline: Individuals who are interested to participate and are deemed eligible for inclusion are invited to a base-line visit which includes assessment of eligibility criteria.

Eligible individuals who provide written informed consent are randomly allocated to one of the two study groups (active snus or placebo snus). The baseline visit will also include medical history, assessment of smoking status (age of initiation of daily smoking, average number of cigarettes smoked per day during the past year, history of previous quit attempts, and intention to participate: "want to quit smoking" versus "want to reduce smoking", history of previous use of NRT or other pharmaceutical or other smoking cessation aids), blood samples, a lung function

test, CO in exhaled air, measurement of blood pressure , height and weight, and Fagerström test of nicotine dependence. At selected study sites the participants will be asked to provide extra 30-50 ml of blood and a sample of buccal cells obtained with a tooth brush, to allow exploratory analyses of adducts. These analyses are not part of this protocol.

The participants are given a diary to register, on a weekly basis, their average daily cigarette and snus consumption during the preceding week, as well as any untoward subjective symptoms should such arise.

Participants are instructed to cut down on smoking by taking a sachet of snus when they feel an urge to smoke. If they still want to smoke after 20-30 minutes, they can do so (although they should then remove the snus sachet to avoid nicotine overdosage). They are also informed that although smoking cessation is preferable, the primary objective of the first 24 weeks of the study is smoking reduction, which in itself is worthwhile since it may have beneficial health effects, and that it might be a first step towards complete smoking cessation.

The number of snus sachets consumed per day is determined by the participants themselves. The fact that one sachet delivers roughly the same amount of nicotine as one cigarette implies that individuals who replace all of their cigarettes with snus will consume about 10-25 sachets per day, that is, the same number as their previous cigarette consumption. The participant is provided with a sufficient quantity of the study product to last until the next visit. Information is given that no other source of nicotine than cigarettes or snus should be used, and that NRT or any other pharmaceutical smoking cessation aid is not allowed.

Week 1: telephone contact to establish preferences for flavors and sachet size (to direct further distribution of study products), to assess compliance and any adverse events, and to monitor each participant's progress.

Clinical visit at week 2 and week 6: Monitoring of each participant's progress including self-reported smoking status according to the participant diary, measurement of CO in exhaled air, blood pressure, and weight (weight only at week 6).

Week 9: telephone contact to monitor each participant's progress, to assess compliance and any adverse events,

Clinical visit at week 12: Clinical visit including measurements of primary and secondary end-Points (based on CO in exhaled air, lung function test, blood tests, self-reported smoking status and snus consumption according to diary, weight, blood pressure, assessment of compliance and adverse events)

Main messages to participants during the first 24 weeks:

"The best thing you can do for your health is to reduce, or preferably quit, smoking…"

"Try to cut down on cigarettes as much as possible by using a sachet every time you feel an urge to smoke, if you still want to smoke after 20-30 min, you can do so, but take out the sachet…"

"Quitting/reducing smoking is difficult; don't feel discouraged if you don't succeed at once…"

Weeks 25-28: Information to participants by mail about test results

Clinical visit at week 30: Monitoring of each participant's progress (including self-reported smoking status and snus consumption according to diary) & measurement of CO in exhaled air, assessment of compliance and adverse events

Main message to participants during 24-48 weeks.

"Quit cigarettes completely by using sachets instead!"

"Since you have been able to substantially reduce your smoking, you should be able to quit completely…"

"Quitting smoking is the best you can do for your health…"

Clinical visit at week 36: Clinical visit including measurements of primary and secondary end-Points (based on CO in exhaled air, lung function test, blood tests, self-reported smoking status and snus consumption according to diary, weight, blood pressure, assessment of compliance and adverse events).

Weeks 37-40: Information to participants by mail about test results

Clinical visit at week 42: Monitoring of each participant's progress (including self-reported smoking status and snus consumption according to diary) & measurement of CO in exhaled air, assessment of compliance and adverse events

Week 48: Clinical visit including measurements of primary and secondary end-

Points (based on CO in exhaled air, lung function test, blood tests, self-reported smoking status and snus consumption according to diary, weight, blood pressure, assessment of compliance and adverse events).

Weeks 49-52: Information to participants by mail about test results

EFFICACY ASSESSMENTS:
Primary Efficacy Assessments:
Smoking status will be assessed at 12 and 24 weeks. "Smoking reduction" at 24 weeks is the primary outcome measure. It is defined as a self-reported reduction of ≥50% compared to baseline in the average number of smoked cigarettes per day during week 20-24, verified by a reduced concentration of carbon monoxide (CO) in exhaled air of at least 1 ppm

Secondary Efficacy Assessments:
Secondary efficacy assessments will be made at 12, 24, 36 and 48 weeks. Secondary end-points include

- "Smoking reduction" at 12 weeks
- Smoking cessation at 12 and 24 weeks defined as self-reported total abstention from cigarettes during the preceding 4-week period verified by a concentration of CO in exhaled air of <10 ppm
- Smoking cessation at 36 and 48 weeks among those who achieved smoking reduction at 24 weeks (cessation here defined as self-reported total abstention from cigarettes during the preceding 4, 12, or 24-week period, verified by a CO-concentration in exhaled air of <10 ppm at all measurements during the specified time period
- Clinical tests and biomarkers at 12 and 24 weeks among all participants, and at 36 and 48 weeks among those who achieved smoking reduction at 24 weeks including body weight, BMI, blood pressure, CO in exhaled air, measures of lung function ($FEV_{1.0}$, FVC, FEV%), total S-WBC, S-CRP, total S-cholesterol, S-HDL, S-LDL, S-fibrinogen, S-cotinine

SAFETY ASSESSMENTS:
Medical history: Prior to randomization the potential participants' medical history will be analyzed to determine eligibility.

Vital signs, Body Weigh and Height: Vital signs will include supine systolic and diastolic blood pressures. Supine recordings will be made after the subject has been recumbent for 3 minutes. Body weight without shoes will be recorded in kg.

Laboratory Parameters: The following laboratory tests are to be performed as indicated by the schedule of assessments:

- Hematology: total S-WBC
- Chemistry: S-CRP, total S-cholesterol, S-HDL, S-LDL, S-fibrinogen, S-cotinine
- Other: CO in exhaled air, lung function tests

At selected study sites an additional 30-50 ml of blood will be sampled from participants at the baseline visit and during follow-up among those who manage to quit smoking. These individuals will also be asked to provide samples of buccal cells which will be obtained with a non-invasive technique similar to brushing the buccal mucosa with a tooth-brush. These samples are intended for exploratory analyses of potential novel biomarkers for cardiovascular disease and cancer risks, e.g. DNA adducts, as well as possible biomarkers for risk of other tobacco-related disease. These exploratory analyses are not part of this clinical protocol so there will be a separate protocol and informed consent form for participants eligible for these analyses.

CO in exhaled air will be analyzed using Micro Smokerlyser EC-50® (Bedfont Scientific Ltd, U. K.). Lung function will be assessed using Easy One Spirometer® (ndd Medizintechnik AG, Zurich, Switzerland) as FEV%, FVC, and $FEV_{1.0}$.

Adverse Events: An adverse event (AE) is any symptom, physical sign, syndrome, or disease that either emerges during the study or, if present at screening, worsens during the study, regardless of the suspected cause of the event. All medical and psychiatric conditions (except those related to the indication under study, that is, nicotine addiction) present at screening will be documented on the Prior Illnesses CRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE CRF during the rest of the study.

AEs may be volunteered spontaneously by the subject, or discovered as a result of general questioning by the study staff. At each visit the subject will be asked, "Have you experienced any problems since your last visit?" All AEs will be recorded on the CRF. For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious AE requiring immediate notification. Follow-up of the AE, even after the date of discontinuation of study products, is required if the AE persists until the event resolves or stabilizes at a level acceptable to the investigator.

In order to avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than the subject's own words. Each AE will also be described in terms of duration, frequency, intensity, association with the study medication, assessment of possible causes, actions taken, and outcome, using choices given on the CRF. Specific guidelines for classifying AEs by intensity and relationship to study medication are given in the tables below.

CLASSIFICATION OF ADVERSE EVENTS BY INTENSITY	
MILD:	The symptom is barely noticeable to the subject and does not influence performance or functioning. Prescription drug treatment is not ordinarily needed for relief of mild AEs but may be given because of personality of subject.
MODERATE:	The symptom is of sufficient severity to make the subject uncomfortable, and performance of daily activities is influenced. Treatment for the symptom may be needed.
SEVERE:	The symptom causes severe discomfort, sometimes of such severity that the subject cannot continue in the study. Treatment for the symptom may be necessary.

CLASSIFICATION OF ADVERSE EVENTS BY RELATIONSHIP TO STUDY MEDICATION	
UNRELATED:	This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY:	This category applies to those AEs that are judged to be unrelated to the test product, but for which no extraneous cause may be found. An AE may be considered unlikely to be related to study product if or when it meets 2 of the following criteria: (1) it does not follow a reasonable temporal sequence from administration of the test product; (2) it could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it does not follow a known pattern of response to the test product; or (4) it does not reappear or worsen when the product is re-administered.
POSSIBLY:	This category applies to those AEs for which a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the product; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the test product.
PROBABLY:	This category applies to those AEs that the investigator feels with a high degree of certainty are related to the test product. An AE may be considered probably related if or when it meets 3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the product; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the product, yet product-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the test product.
DEFINITELY:	This category applies to those AEs that the investigator feels are incontrovertibly related to test product. An AE may be assigned an attribution of definitely related if or when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the product; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to product (if rechallenge occurs); and (4) it follows a known pattern of response to the test product.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

Serious Adverse Events: A serious adverse event (SAE) is defined as any AE that meets one or more of the following criteria:

- The event is fatal or life-threatening.
- The event is permanently disabling (incapacitating or interfering with the ability to resume usual life patterns).
- The event results in unplanned inpatient hospitalization or prolongation of existing hospitalization.
- The event is a congenital anomaly.
- The event requires medical intervention of any kind in order to prevent any of the aforementioned outcomes.

A death occurring during the study or within 1 week of stopping the treatment must be reported to the trial safety coordinator. A serious AE is not necessarily severe; for example, an overnight hospitalization for a diagnostic procedure must be reported as a serious AE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours' duration may be rated as severe but may not be considered serious.

Any serious adverse event due to any cause that occurs during the investigation, whether or not related to the study medication, must be reported within 24 hours of occurrence or when the investigator becomes aware of the event. The investigator must send a preliminary report of any SAE encountered during the study and for 1 month after a subject has discontinued or completed the study to the trial safety coordinator by fax within 24 hours using an SAE Report Form. The event must also be recorded on the standard AE CRF. Preliminary reports of SAEs must be followed by detailed descriptions later on, including clear photocopies of hospital case reports, consultant reports, autopsy reports, and other documents when requested and applicable. SAE reports must be made whether or not the investigator considers the event to be related to the investigational product.

Appropriate remedial measures should be taken to treat the SAE and the response should be recorded. Subjects must be closely followed until sufficient information is obtained to indicate a return to normal status or until the event stabilizes at a level acceptable to the investigator. Clinical, laboratory, and diagnostic measures should be employed as needed in order to determine the etiology of the problem. The results will be reported promptly to the sponsor.

Other Significant Adverse Events: To ensure subject safety, the investigator should also notify the safety coordinator should any AE occur that is considered significant but does not meet criteria for an SAE, or that is considered unexpected. In addition, any field monitor who notes a significant AE or medical condition while reviewing the CRFs or source documents at the site must immediately convey this information to the trial safety coordinator.

3.4.3 Appropriateness of Measurements

All assessments to be used in this study are common, standard measurements frequently used in smoking reduction/cessation studies.

4. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Completed CRFs for this study will be forwarded to the sponsor's representative where editing and construction of a quality-assured database will occur. There will be no interim analyses of treatment efficacy.

4.1 Determination of Sample Size

To reliably detect ($p<0.05$, statistical power >80%) a more than two-fold increase in the odds of achieving smoking reduction at 24 weeks among the active versus placebo groups, and assuming a smoking reduction rate of 15% in the placebo group versus 25% in the active snus group (corresponding to an odds ratio of 1.9), the target sample size can be estimated at 250 per group, that is, a total size of 500 study participants.

In previous randomized studies of NRT versus placebo, NRT has resulted in an increased proportion of participants achieving short- to medium term smoking cessation with odds ratios in the order of 1.5-3. Epidemiological, cross-sectional studies from Sweden have indicated that snus might be more effective as an aid in smoking cessation than NRT. Against this background the mentioned target sample size should provide reasonable statistical power for current purposes.

4.2 Study Population

The statistical analysis will include all randomized, eligible subjects on an "intention to treat" (ITT) basis. Exploratory analyses will also be done on those who achieved "smoking reduction" at 24 weeks.

4.3 Background and Demographic Characteristics

The population will be described in terms of age, gender, previous smoking history & quit attempts, and use of NRT or other pharmaceutical smoking cessation aids, Fagerström score, medical history, and other relevant data collected at baseline.

4.4 Efficacy Analysis

4.4.1 Primary Efficacy Variable

The primary end-point, that is, the proportion achieving "smoking reduction" at 24 weeks, will be analyzed with logistic regression modeling among eligible subjects using intent-to treat criteria and with participants who terminated the study prematurely for any reason considered to be failures.

4.4.2 Secondary Efficacy Variables

Secondary end-points will be analyzed using parametric or non-parametric standard statistical methods. The proportion achieving "smoking reduction" or "smoking cessation" at the times and with the criteria specified in section 3.4.2 (Description of Assessments), will be analyzed with logistic regression modeling among eligible subjects using intent-to treat criteria and with participants who terminated the study prematurely for any reason considered to be failures. Exploratory analyses of secondary end-points will also be done on those who achieved "smoking reduction" at 24 weeks.

A priori hypotheses concerning secondary end-points include that allocation to active snus will result in beneficial effects on lung function and cardiovascular biomarkers. Such effects are hypothesized to be directly related to the level of smoking reduction. Overall nicotine consumption (as measured by S-cotinine) is not expected to increase in the active snus group.

5. STUDY MANAGEMENT

5.1 Approval and Consent

5.1.1 Regulatory Guidelines

The study will be performed in accordance with local national laws (as applicable), the guidelines of the International Conference on Harmonisation (ICH), and the guidelines of the Declaration of Helsinki adopted by the 18th World Medical Assembly in Helsinki, Finland in 1964 and amended by subsequent assemblies in Tokyo, Japan in 1975; Venice, Italy in 1983; Hong Kong in 1989; Somerset West, South Africa in 1996, and in Edinburgh, Scotland, October 2000.

5.1.2 Research ethics and approval by IRB/IEC

Cigarette smoking is a significant public health problem in Serbia. The prevalence of daily smoking in Serbia is one of the highest in Europe. Serbian health authorities are aware of the problem and have started to take measures against smoking. However, the high smoking prevalence, low public awareness of the health hazards with smoking, and economic problems to fund smoking reduction/cessation programs, represent a significant challenge to all anti-smoking measures in Serbia. In addition, pharmaceutical smoking cessation aids such as NRT are generally prohibitively expensive. In addition, the benefit with such therapies long-term remains modest. In conclusion, there is a great need for further research on effective strategies for smoking reduction/cessation in Serbia.

The current trial aims to determine the acceptability of Swedish snus in a setting without a long cultural tradition of oral tobacco products, as well as to evaluate the efficacy of snus for smoking reduction/cessation. The trial thus has considerable interest both from a scientific and public health point of view.

It might be viewed as problematic from an ethical point of view that the study does not entail treatment with products that have been demonstrated to be effective to achieve smoking reduction/cessation in the context of controlled clinical trials, such as, NRT, bupropion or varenicline. However, there are extensive epidemiological data from Sweden suggesting that snus has been used by many smokers to quit smoking and that it might even be more effective than NRT in achieving complete, long-term smoking cessation.

There are extensive data from epidemiological studies demonstrating that smokeless tobacco, particularly low-nitrosamine Swedish snus, is associated with dramatically reduced health risks compared to cigarette smoking. The risk profile with snus thus appears closer to that of no tobacco use, than to cigarette smoking. So, switching from cigarettes to snus, albeit another tobacco product can be expected to be associated with significantly reduced health risks and thus harm reduction.

The addiction to cigarettes may not entirely be a result of the physical addiction to nicotine, but also in part a psychological phenomenon related to cues and attributes of cigarette smoking. It is therefore essential to include a placebo control arm in studies of smoking cessation, and to conduct such trials with a randomized, double-blind technique, even though such study features may be viewed as problematic from an ethical point of view.

The clinical tests in the trial involve invasive methods (blood sampling), but such tests are part of routine medical care and are associated with minimal risks. Individual test results will be treated

confidentially and will only be revealed to the study participants to minimize problems related to personal integrity. All participants will provide written informed consent to participate in the trial.

For participants who have managed to quit smoking with snus and who wish to continue with snus after completion of the study, the sponsor intends to make snus available from commercial retailers within the city of Belgrade, unless this is made impossible by regulatory or other circumstances outside of the sponsor's control.

The participants will be given small gifts at the clinical visits involving blood tests as a token of appreciation on the part of the study team. However, the value of these gifts will not exceed 5-10 Euro so there is no reason to believe that these gifts will act as a pressure on the participants to continue in the trial should they wish to terminate their participation.

Against this background, it can be concluded that the scientific value of this study by far outweighs the ethical problems which in general are only of minor significance.

Conduct of the study will be approved by an appropriately constituted institutional review board (IRB) or independent ethics committee (IEC). Approval is required for the study protocol, protocol amendments, informed consent forms, subject information sheets, and advertising materials. No study products will be shipped to a site until written IRB/IEC authorization has been received by the sponsor or its representative. The international coordinating investigator in collaboration with the investigators at each study site will be responsible for obtaining such authorization from the appropriate IRB/IEC.

5.1.3 Informed Consent

For each trial subject, written informed consent will be obtained prior to any protocol-related activities. As part of this procedure, the investigator or one of his or her associates must explain orally and in writing the nature, duration, and purpose of the study, and the action of the study products in such a manner that the subject is aware of the potential risks, inconveniences, or adverse effects that may occur. An English version of the written information to be used is attached to this protocol. This written information remains tentative until approved by the relevant IRB.

5.2 Discontinuation of the Study by the Sponsor

The sponsor reserves the right to discontinue the study at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Should the study be terminated and/or the site closed for whatever reason, all documentation and equipment pertaining to the study must be returned to the sponsor or its representative.

5.3 Study Documentation

By signing page 2 of this protocol, the investigator acknowledges that he/she has received appropriate information about the products being tested in the trial and assures the sponsor that he/she will comply with the protocol. No changes in this protocol can be made without the sponsor's written approval.

The investigator will supply the sponsor with:

- Curricula vitae for all investigators involved in the trial
- Signed protocol signature page

The sponsor will supply the investigator with:

- Clinical study protocol
- Other relevant information about the study products
- Sample informed consent form
- Case report forms (CRFs)/instruction manual
- Equipment for clinical measurements of CO in exhaled air and lung function

5.4 Study Monitoring and Auditing

This study will be monitored at all stages of its development by the clinical research personnel employed by the sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to protocol and in order to comply with guidelines of Good Clinical Practice. On-site review of CRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each subject.

As this is a trial only including healthy volunteers there are no clinical records on the participants. Therefore, the CRF will serve as the main source document in this trial, although a variety of documents including laboratory reports, patient diaries, and printouts from medical equipment also will serve as source documents.

Medical advisors and clinical research associates or assistants may request to witness subject evaluations occurring as part of this protocol. The investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the sponsor to assure acceptable protocol execution. The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required subject records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives, and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in CRF generation, where clinically appropriate.

5.5 Retention of Records

The investigator must arrange for retention of study records at the site for a period of 15 years. The nature of the records and the duration of the retention period thus meet the requirements of regulatory authorities within the European Union. The investigator should take measures to prevent accidental or premature destruction of these documents.

5.6 Use of Study Findings

By signing the study protocol, the investigator agrees to the use of results of the study for the purposes of national and international regulatory affairs or registration. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement. Reports of the study will be prepared by the international coordinating investigator in collaboration with the sponsor or its representative.

5.7 Publications

As a multicenter trial, the sponsor and the investigators intend that the data from all centers participating in the trial will be published in the form of a joint report. A publication committee selected by the international coordinating investigator will submit draft manuscripts to all

participating investigators for their comments. In conformity with the uniform requirements for manuscripts submitted to biomedical journals published by the International Committee of Medical Journal Editors (see discussion in Kassirer & Angell, 1991); investigators whose contribution consists solely in the collection of data will not be named individually as authors. Rather, those investigators will be identified and acknowledged in a note.

Individual investigators and/or their associates subsequently may publish additional findings of this study, i.e. results not pertaining to the randomized smoking reduction/cessation comparison, in scientific journals or present them at scientific meetings, provided that the sponsor is given ample opportunity to review any proposed abstract, manuscript, or slide presentation prior to its submission. This review is required to ensure that the sponsor is aware of all written and oral presentations of the data and does not imply any editorial review or restriction of the contents of the presentation or use.

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7. APPENDICES

- Detailed description of study products
- Flow chart of scheduled events
- Sample of written informed consent form
- Sample of texts to be used for advertisements and/or posters to recruit participants
- The Fagerström test
- Sample participant diary