The US and Serbian randomized clinical trials on the role of Swedish snus for long term smoking cessation. Systematic review and meta-analysis of the available experimental evidence.

1.1 Abstract

Several correlational lines of evidence from both Sweden and Norway suggest that snus can aid in smoking cessation. In both countries, snus is the most commonly reported smoking cessation product, and appears to be associated with better long term cessation rates than NRT.

Although these observations are encouraging, the lack of experimental data based on randomized clinical trials has been cited as problematic given the methodological limitations of cross-sectional survey data. Scientists and health agencies have asked for randomized clinical trials. Against this background, Swedish Match entered into discussions during 2004-2005 with academic researchers with an interest in conducting smoking cessation trials that would test the efficacy of snus among smokers motivated to quit or substantially reduce their smoking. These deliberations led the company in 2006 to accept sponsorship for two randomized trials, one in Serbia and one in the US.

As there is no internationally accepted governance structure specific for clinical studies of tobacco products, it was agreed before the trials were initiated that the governance and conduct of the two randomized trials should be as similar as possible to accepted procedures for controlled trials of pharmaceutical products.

The Serbian and US studies were both randomized, placebo-controlled, double blind clinical trials that tested whether *ad lib* provision of Swedish snus as compared to placebo affects subsequent smoking habits. The US study was conducted in five sites in the USA and Serbian study at two centers in Belgrade, Serbia.

The snus products were manufactured according to the GothiaTek® standard. In the Serbian trial the participants could choose from two different pouch sizes (0.5 g and 1 g) and two different flavors. In the US trial participants could choose from two different pouch sizes (0.5 g and 1 g), but all study products had the same flavoring. The placebo snus products were almost identical to the snus products in physical appearance, mouth feel, pH, flavoring, and other sensory characteristics but did not contain tobacco or nicotine. All study products were supplied in identical, food-grade plastic containers, and were only identified by a unique serial number that permitted traceability. Use of study products was *ad libitum* and no minimum number of pouches per day was defined.

The studies involved subjects in a similar age range (USA: 25-65 years; Serbia: 20-65 years). The studies were restricted to subjects who had smoked daily for >1 year, and collected self-reported tobacco status data in a diary completed by the subject, and recorded exhaled carbon monoxide (CO) levels at the clinical visits.

The US study involved a screening period of two weeks, a study product test period (weeks 1 to 4 post-randomization), an intervention phase (weeks 5 to 16) during which subjects were encouraged to stop smoking completely, and a follow-up phase (weeks 17 to 28). Study products were supplied only up to week 16, with tapering of product use during weeks 14 to 16. The objectives of the US study included comparison of biologically verified continued quit rates during weeks 6 to 28, and 1-week point prevalence quit rate at weeks 16 and 28.

The Serbian study involved a smoking reduction stage (weeks 1 to 24 post-randomization) and a smoking cessation stage (weeks 25 to 48). While the primary objective of the Serbian study related to smoking reduction at week 24, other objectives related to both continued and point prevalence rates of biologically verified smoking cessation.

Measurement of abstinence, biochemical verification, and statistical analyses was done according to recommendations by the Society for Research on Nicotine and Tobacco. Missing responses or missing data related to smoking were thus interpreted as though the subject had smoked on that occasion. Analyses of efficacy were on the basis of "intention to treat" and no randomized subject was excluded.

A total of 319 participants entered the Serbian study during January, 2008 through April, 2009. Few participants had previous exposure to nicotine replacement therapy or other pharmaceutical cessation aids.

The mean amount of study product used per day among those allocated to snus was moderate: the weekly average ranged from 3.5 to 4.7 g per day, but tended to increase over time. Those allocated to the placebo group had a marginally higher consumption.

At the week 24 visit there was no difference between the snus and placebo group in the proportion who achieved the protocol definition of a $\geq$50% smoking reduction, but a higher proportion of participants in the snus group reported >75% reduction of their average number of smoked cigarettes per day compared to baseline, particularly during the first six months of the trial: at week 24 the this proportion was 9.5% in the snus group compared to 2.5% in the control gropup ($p<0.01$).

The number of participants with CO confirmed smoking abstinence during the preceding 4, 12, and 24 week period was higher in the snus group compared to the placebo group at both the week 36 and week 48 visit. The estimated odds ratios ranged from 2.1 to 3.3, but only the estimate for 12-week continued abstinence at week 48 was statistically significant ($p<0.05$).

The number of participants with CO confirmed 7 day point prevalence abstinence was higher in the snus group compared to the placebo group at the clinical visits week 12, 24, 36, and 48. The estimated odds ratios (snus versus placebo group) ranged from 1.9 to 3.4, but only the estimate at 36 weeks was statistically significant.

A total of 250 subjects were included in the US trial during February through August, 2009. They were randomly allocated receive either snus or placebo (125 each). About half of the participants reported previous exposure to NRT, and nearly two thirds had tried other pharmaceutical smoking cessation products.

As in Serbia, study product usage in the US trial was relatively moderate.

Biologically verified, continuous abstinence during week 6 through 28 was 4.0% and 1.6% for snus and placebo, respectively. Statistically significant advantage for snus over placebo occurred for point-prevalence outcomes at weeks 6 and 16 ($p<0.05$).

In summary, biologically verified smoking cessation rates in the two trials were generally 2-3 times higher among subjects allocated to snus, but the overall low level of cessation precluded firm conclusions due to lack of statistical precision.

In both studies, using snus was safe and generally well tolerated. However, treatment-related adverse events (AEs) were reported more frequently by participants allocated to snus compared to placebo, but they were mostly classified as mild. The type of AEs that occurred more frequently in the snus groups typically concerned symptoms related to nicotine exposure (tachycardia, nausea, increased salivation, vomiting, and hiccups).

The main strength of the two trials was their double-blind, placebo-controlled design. The main weakness was that none of the study centers had previously been involved in smoking cessation programs or worked with either pharmaceutical or behavioral cessation interventions which may have affected the overall level of complete cessation.

1.2 Background

Snus has never been marketed in Scandinavia as a smoking cessation aid or for tobacco harm reduction purposes. It has been marketed as a traditional Swedish tobacco product under historic brand names many of which go back more than a century (Rutqvist, Curvall, Hassler, Ringberger & Wahlberg, 2011). However, observational data from Scandinavia indicate that many smokers have switched from smoking cigarettes to using Swedish snus (Ramström & Foulds 2006, Lund, McNeill, Scheffels 2010, Lund, Scheffels & McNeill 2011, Rutqvist 2013). Among current snus users with a previous history of smoking, daily dual use of both cigarettes and snus is infrequent. Such data indicate that many smokers have quit smoking completely by switching to snus which is part of a phenomenon sometimes referred to as the "Swedish Experience" that started in the late 1960s-early 1970s.

In Scandinavia like in many other countries most successful smoking cessation attempts are accomplished unassisted, but there is also an extensive use of various cessation aids including pharmaceutical products. However, snus is the most commonly reported cessation aid in both Sweden and Norway, particularly among males. Use of snus at the latest quit attempt has been reported to be associated with a higher long-term success rate compared to NRTs in terms of complete cessation. (Ramström & Foulds 2006, Lund, McNeill, Scheffels 2010, Lund, Scheffels & McNeill 2011, Rutqvist 2013).

The mentioned conclusions about the role of snus for smoking cessation are based on observational data typically derived from cross-sectional surveys. One methodological weakness of such data is that the collected information is based on self-reports and non-smoking status is typically not verified biochemically. Also, although observational data can establish temporal relationships, such as a switch from cigarettes to snus on an individual level, causal inferences may be difficult. Some smokers who switched to snus may theoretically have stopped using any tobacco product if snus had not been available.

Observations in population surveys on reported quitting strategies and cessation outcomes are probably also to some extent biased by self-selection. Smokers with low nicotine dependence may more often choose to quit unassisted and succeed in remaining smokefree than smokers with high nicotine dependence. Among smokers who elect to use some form of cessation aid, self-selection between different aids is probably also a concern. Some individuals may experience oral problems with snus (possibly related to the products relatively high pH), and some simply do not favor snus for esthetic or other reasons. Such individuals are unlikely to try snus for smoking cessation purposes. Self-selection mechanisms may theoretically be related to the long-term outcome of quit attempts. These considerations imply that one should be cautious with extrapolating conclusions based on survey data about the relative efficacy of different cessation strategies to the total population of smokers.

The mentioned limitations may help to explain why the role of snus for smoking cessation has remained controversial despite the seemingly compelling epidemiology.

In the SCENIHR report on smokeless tobacco published in 2008 (SCENIHR Committee 2008), which was commissioned by the European Union Health Authority, it was noted that snus has been used more often than pharmaceutical nicotine products by males in Sweden as an aid to stop smoking, and that available data are consistent in demonstrating that male snus users are more likely to quit smoking than non-users. The report concluded that several retrospective studies, suggested results with snus to be on par or above those achieved with

nicotine replacement products. But the report also stated that the switch from cigarettes to snus that has occurred in Sweden may reflect cultural factors that are specific to Swedish males and that it is not possible to extrapolate future tobacco use patterns across countries due to societal and cultural differences. The report cited the absence of information based on randomized clinical trials of snus and concluded: *"in the absence of such evidence it is not possible to draw reliable conclusions as to the relative effectiveness of smokeless tobacco as an aid to clinical smoking cessation in comparison with either placebo or other established therapies".*

The cited need for experimental data on snus has stimulated several European research groups to look into the prospects for conducting randomized clinical trials of snus as a smoking cessation aid. This interest is enhanced by the fact that the ability of a modified risk tobacco product to increase complete quit rates among current smokers may constitute a cornerstone in tobacco harm reduction scenarios based on the availability and increased use of such a product. It may be hypothesized that dual use, that is, only partial substitution of cigarettes among smokers with a reduced harm product, may not result in appreciable health benefits. In contrast, complete smoking cessation through a switch to snus has been estimated to result in health benefits that approach those of unassisted quitting (Gartner et al 2007).

In 2004-2005 Swedish Match entered into discussions with academic researchers with an interest in conducting smoking cessation trials that would test the efficacy of snus. The deliberations led the company to accept sponsorship for two randomized trials, one in Serbia and one in the US. Early on in the discussions, governance issues and credibility of the eventual trial data were prominent concerns, given the wide-spread skepticism within the tobacco control community regarding research conducted or sponsored by tobacco companies (IOM Committee Report on MRTP Applications, 2011). However, before a governance structure had been put in place for the trials, two feasibility studies were done, one in Serbia and one in the US.

1.3 Feasibility studies

As these preliminary studies were done before a governance structure was fully developed and implemented, Swedish Match makes no claims on the basis of the reported findings. The outcome of the feasibility studies is simply mentioned as background information to the designs that eventually were chosen for the two trials.

Serbia – In 2004-2005 researchers at one of the centers in Belgrade that eventually participated in the randomized trial (an occupational health center at the head office of the Nis-Jugopetrol Corporation) conducted a study aimed at elucidating the feasibility of a long-term smoking cessation trial in Serbia involving Swedish snus. Such a pre-study was considered necessary given the fact that there is no traditional use of any type of oral tobacco product in Serbia. The study goals included to assess the acceptability of snus products, particularly with respect to taste preferences among Serbian smokers. A nicotine patch was used as a comparator (Nicorette®, 15 mg). A total of 39 smokers (average age 42.1 years) agreed to participate after being informed about the study, the procedures and nature of the tested products. They were recruited among subjects undergoing routine health check-ups. A total of 21 were randomly assigned to test snus products, and 18 to test the nicotine patch. The average daily cigarette consumption among those allocated to snus was

27.6 vs 27.8 among those allocated to the nicotine patch. Participant disposition according to allocated product and Fagerström score is shown in Table 1. Subjects allocated to snus tested different brands of Swedish snus (1.0 g pouches) manufactured according to the GothiaTek® standard including brands with characterizing flavors (eucalyptus and licorice) and a traditional Swedish brand with no such flavor. Study duration was one month. The baseline evaluation included smoking history, average number of cigarettes smoked per day, the Fagerström test for nicotine dependence, and measurement of carbon monoxide in exhaled air (CO), body weight, and resting systolic and diastolic blood pressure. Participants were instructed to reduce or preferably quit smoking with the help of their allocated product although no specific target quit date was set. Telephone contacts were scheduled once per week.

Outcome after one month was evaluated based on self-reported smoking and measurement of weight, blood pressure and CO in exhaled air. The participants allocated to snus were also interviewed about their brand preferences.

Both male and female participants considered the flavored snus brands acceptable, whereas none preferred the traditional brand. Snus-allocated subjects showed a substantial reduction in CO-levels at the one month follow-up which was comparable to that achieved by participants allocated to the nicotine patch (Table 2). One out of 21 subjects (5%) in the snus group reported having stopped smoking completely, six reported use of snus products alone (29%), six combined snus with cigarettes (29%), and eight (38%) had ceased using snus and continued with cigarettes alone. In the nicotine patch group four out of 18 subjects (22%) reported having stopped smoking, seven (39%) continued to smoke concomitant with patch use, and seven (39%) reported using cigarettes alone. There were no appreciable changes compared to baseline in average resting systolic and diastolic blood pressure, or body weight among participants in either the snus or nicotine patch group (data not shown). Poor motivation among some participants to comply with study procedures was cited as a major issue by the responsible trialist.

The conclusion from this short-term pilot project was that a long-term smoking cessation trial involving Swedish snus might be possible in Serbia provided the study design excluded smokers unmotivated to quit and if properly flavored products were used.

US – A feasibility study was conducted by an external contractor (Fathom Research) during 2006 at two sites: Charlotte, NC, and San Diego, CA. The objectives of the study included to test the acceptability of different brands of pouched Swedish snus among habitual smokers. The tested brands differed with regard to flavor (mint and wintergreen, and a traditional product with no characterizing flavor), pouch size (1.0 g and 0.5 g), and pouch color (white, brown, and black). An additional goal was to assess potential psychological barriers among the subjects to participate in a long-term, smoking cessation trial involving use of a smokeless tobacco product.

A total of 49 adult smokers (35 male, 14 female) were recruited to a 45 minute interview and testing of snus products. During the interview product concept and use were explained, and acceptable and optimal brand preferences were identified. Product was placed for in-home trial with 43 subjects who were asked to keep a log of snus use and smoking for two weeks. During that time they replaced some of their cigarettes with snus products. Two, 15-minute call back interviews were conducted after the trial period during which the following dimensions were checked: acceptability in normal use situations, pouch size preference, and possible sensory related compliance risks.

The study showed that white pouches were preferred by nearly all respondents. Mint flavored products were well accepted, and often preferred to the other products. Wintergreen flavor was polarizing, and the traditional brand with no characterizing flavor was the least preferred brand. When given a choice before trial, most respondents wanted to try the smaller size as it was perceived as easier to use and more discreet, particularly among women. However, most respondents said they understood that the larger size would be more effective, delivering more nicotine.

Identified psychological barriers included perceptions of the products as conventional “dip”, as just another tobacco product that is just as dangerous as smoking, and fears related to ease & comfort of use, and spitting.

1.4 Governance of studies

As there is no internationally accepted governance structure specific for clinical studies of tobacco products, or for trials sponsored by a tobacco company, it was agreed before the randomized studies were initiated that the governance and conduct of the two trials should be as similar as possible to accepted procedures for controlled clinical trials of pharmaceutical products. The governance structure finally agreed on thus included the following elements:

- Protocols developed in collaboration between the individual research teams and the sponsor according to internationally accepted guidelines
- Studies 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
- Written, full informed consent from all study participants
- Conduct of study approved by an appropriately constituted institutional review board (IRB) or independent research ethics committee (IEC)
- Trials conducted according to full ICH-GCP (“Good Clinical Practice”)
- Management of all clinical and other study-related information, including monitoring, conducted by internationally well-reputed Contract Research Organizations (CRO:s) with extensive experience of controlled clinical trials of pharmaceutical products (Serbian study: i3 Research, US study: Covance)
- All data handling and statistical analyses to be conducted by external contractors according to pre-specified statistical analysis plans (Serbian study: i3 Statprobe, US study: Covance)
- Prospective registration of the trials at www.clinicaltrials.gov
- Commitment by the sponsor to publish results irrespective of trial outcomes
- Publication in peer-reviewed scientific journals according to the CONSORT guidelines (http://www.consort-statement.org)
- Commitment to make individual study data available for systematic reviews and/or meta-analyses conducted according to internationally accepted guidelines (e g the PRISMA guidelines, http://www.prisma-statement.org).

1.5 Rationale for study designs

As there were no previous controlled trials of snus it was considered reasonable to use a placebo-comparator as this design would generate direct information about the efficacy of snus. It was also considered reasonable to conduct the studies double-blind as the double-blind, placebo-controlled approach is considered to be the gold standard for evaluating clinical interventions, and is typically the first step to establish efficacy. However, this design precluded the inclusion of a second, orally administered comparator, for instance, nicotine gum or lozenges. The use of snus products would interfere with the participant's ability to chew gum or use other oral products, and vice versa. Theoretically, it might have been possible to include a nicotine patch as an additional comparator, although a placebo-controlled, double-blind study design including snus, placebo snus, nicotine patch, and a placebo patch would have implied significant challenges in terms of study product logistics.

Since use of NRT is quite prevalent among smokers who want to quit in the US, it was expected that a substantial proportion of the participants in the US study would have a history of previous unsuccessful quitting attempts with NRTs. It would then be possible to assess the relative efficacy of snus among those with a previous history of NRT exposure versus those without. Information on possible cross resistance between snus and NRT ("Does snus work among smokers who have failed on NRT?") might be considered as clinically more relevant than a direct comparison of efficacy with an NRT ("Is snus more or less efficacious than NRT?"). In the Serbian trial, on the other hand, it was expected that few participants would have tried NRT or other pharmaceutical cessation aids because the cost of such products is typically prohibitive for most Serbian smokers.

The design of the US trial entailed a relatively short period (16 weeks) of active treatment during which participants were issued study products. Thereafter, subjects were instructed to refrain from nicotine-containing products (unless there was an imminent danger of smoking relapse among those who had managed to quit). This design mimics that typically used in many previous randomized trials of NRT products where the objective is not only to promote smoking cessation but also to treat the participants' dependence to nicotine (Silagy et al. 2007).

In the Serbian trial the primary outcome variable during the first 6 months was smoking reduction. It was hypothesized that recruitment to a smoking cessation program may be more successful if the proposed goal is to reduce smoking rather than total cessation. Smokers who have made previous unsuccessful quit attempts might abstain from participating in a program if the requirement is immediate, total abstention. Initial smoking reduction may facilitate complete cessation later on (Asfar, Ebbert, Klesges, Relyea 2011).

Only those participants who were found to have substantially reduced their smoking at the week 24 visit were actively followed up to 48 weeks. During weeks 24-48 the main objective was complete cessation. Study products were distributed throughout the study period with no prescribed tapering after a specified time point. The aims of the trial thus focused on smoking cessation but did not include treating the participants' nicotine dependence. The Serbian design can be described as being naturalistic because clinical experience from Scandinavia indicates that smokers who use snus as a smoking cessation aid typically do not switch abruptly from cigarettes to snus. The transition period of dual daily use can last from weeks to many months. Many successful quitters continue to use snus long term (Giljam & Galanti 2003).

1.2 Background

Snus has never been marketed in Scandinavia as a smoking cessation aid or for tobacco harm reduction purposes. It has been marketed as a traditional Swedish tobacco product under historic brand names many of which go back more than a century (Rutqvist, Curvall, Hassler, Ringberger & Wahlberg, 2011). However, observational data from Scandinavia indicate that many smokers have switched from smoking cigarettes to using Swedish snus (Ramström & Foulds 2006, Lund, McNeill, Scheffels 2010, Lund, Scheffels & McNeill 2011, Rutqvist 2013). Among current snus users with a previous history of smoking, daily dual use of both cigarettes and snus is infrequent. Such data indicate that many smokers have quit smoking completely by switching to snus which is part of a phenomenon sometimes referred to as the "Swedish Experience" that started in the late 1960s-early 1970s.

In Scandinavia like in many other countries most successful smoking cessation attempts are accomplished unassisted, but there is also an extensive use of various cessation aids including pharmaceutical products. However, snus is the most commonly reported cessation aid in both Sweden and Norway, particularly among males. Use of snus at the latest quit attempt has been reported to be associated with a higher long-term success rate compared to NRTs in terms of complete cessation. (Ramström & Foulds 2006, Lund, McNeill, Scheffels 2010, Lund, Scheffels & McNeill 2011, Rutqvist 2013).

The mentioned conclusions about the role of snus for smoking cessation are based on observational data typically derived from cross-sectional surveys. One methodological weakness of such data is that the collected information is based on self-reports and non-smoking status is typically not verified biochemically. Also, although observational data can establish temporal relationships, such as a switch from cigarettes to snus on an individual level, causal inferences may be difficult. Some smokers who switched to snus may theoretically have stopped using any tobacco product if snus had not been available.

Observations in population surveys on reported quitting strategies and cessation outcomes are probably also to some extent biased by self-selection. Smokers with low nicotine dependence may more often choose to quit unassisted and succeed in remaining smokefree than smokers with high nicotine dependence. Among smokers who elect to use some form of cessation aid, self-selection between different aids is probably also a concern. Some individuals may experience oral problems with snus (possibly related to the products relatively high pH), and some simply do not favor snus for esthetic or other reasons. Such individuals are unlikely to try snus for smoking cessation purposes. Self-selection mechanisms may theoretically be related to the long-term outcome of quit attempts. These considerations imply that one should be cautious with extrapolating conclusions based on survey data about the relative efficacy of different cessation strategies to the total population of smokers.

The mentioned limitations may help to explain why the role of snus for smoking cessation has remained controversial despite the seemingly compelling epidemiology.

In the SCENIHR report on smokeless tobacco published in 2008 (SCENIHR Committee 2008), which was commissioned by the European Union Health Authority, it was noted that snus has been used more often than pharmaceutical nicotine products by males in Sweden as an aid to stop smoking, and that available data are consistent in demonstrating that male snus users are more likely to quit smoking than non-users. The report concluded that several retrospective studies, suggested results with snus to be on par or above those achieved with

Clinical trials of smoking cessation have typically involved participation from dedicated smoking cessation centers with expertise in such interventions including cessation counseling. Smokers seen at such centers typically have a high motivation to quit smoking, and include smokers who have been referred to the center for help with cessation. In both the US and in Serbia attempts were made to recruit such dedicated centers but these efforts eventually failed for various reasons. The reluctance on the part of academic institutions to participate in studies sponsored by a tobacco company may have been a contributing factor. This implied that those centers that eventually did participate had no previous clinical experience with smoking cessation interventions. In addition, with the exception of one of the Serbian trialists, none of the researchers at the participating centers had previous experience with Swedish snus.

Serbian investigator site audit - When accrual of participants to the Serbian trial started in January 2008, it was intended that a third center would participate in the study: an occupational health center at the Serbian Railway Health Institute. After a few months, the monitoring staff at i3 Research reported major compliance issues at this center. As a result, a sponsor-initiated formal Investigator Site Audit was done at the center during September 2008. The audit was conducted by an outside contractor. The Audit Plan and Report are enclosed in Appendix 1b.

In summary, the audit revealed several major problems including lack of evidence that participants had adequately consented to participate in the study, failure to comply with the investigational plan, inadequate reporting of adverse events, and failure to respond to the monitor's instructions at monitoring visits and in follow-up letters. Most of the issues revealed during the audit had already been detected by the monitor and discussed with the investigator, but corrective actions had not or only in part been implemented by the investigator, even though the site had been re-trained on several occasions. None of the participants at the site had reached week 24, that is, the time for the evaluation of the trial's primary end-point. The auditor recommended that the site should not continue to recruit any further participants.

On the basis of the audit findings, and after consultation with the monitor, the sponsor (Swedish Match) decided in October 2008 that the site should not recruit further participants, that all trial related activities be stopped for subjects already included, and that no data from this center should be part of any analyses of the trial.

1.8 Study designs

Detailed information on study design and procedures are included in the study protocols (Appendix 1a and 2a), and in the published trial reports (Appendix 1f and 2e). In summary, the Serbian (SM 07-01) and US trial (SM 08-01) were both randomized, placebo-controlled, double blind clinical trials that tested whether *ad lib* provision of Swedish snus as compared to placebo affects subsequent smoking habits.

The US study was conducted in five sites in the USA (Daytona Beach, CA, Austin, TX, Dallas/Fort Worth, TX, Portland, OR, and Evansville, KY). The Serbian study was conducted in two centers in Belgrade, Serbia. The US sites were Covance clinical research centers focused on conducting phase 1-4 clinical trials, mostly of pharmaceutical products. The Serbian sites were occupational health care centers located at a major Serbian corporation (Nis-Jugopetrol) and at a national nuclear science research center (Vinca Institute). Accrual

of potential participants in both the US and Serbia was through advertising in local media and word-of mouth. In the US recruitment was also done from a database kept by Covance of individuals potentially willing to participate in their clinical trials. The character of the centers implied that there were no referrals of smokers from other centers.

Both studies involved subjects in a similar age range (USA: 25-65 years; Serbia: 20-65 years). The studies were restricted to subjects who had smoked daily for >1 year, with a similar minimum average consumption level in the preceding month (USA: 9 cigs/day; Serbia: 10 cigs/day), and to subjects who were in good health and who were motivated to change their smoking habits (USA: motivated to quit smoking by a smokeless tobacco (ST) product; Serbia: motivated to substantially reduce or quit smoking). Both studies also effectively excluded current users of ST, in the US by a specific exclusion criterion, in Serbia by ST not being available on the market. Both studies also excluded subjects who had used any type of pharmaceutical or other product for smoking cessation in the preceding three months, who had oral conditions that could be worsened by treatment, who abused alcohol or drugs, who had a history of cardiovascular disease, and who were pregnant or lactating. Both studies collected self-reported tobacco status data in a diary completed by the subject, and recorded exhaled carbon monoxide (CO) levels at intervals, as well as conducting a Fagerström test for nicotine dependence.

The US study involved four periods, a screening period of two weeks to evaluate eligibility, a study product test period (weeks 1 to 4 post-randomization) during which the subjects were instructed to use the study products when they felt an urge to smoke, initially without any requirement for complete abstention from cigarettes, an intervention phase (weeks 5 to 16) during which subjects were encouraged to stop smoking completely and to use their allocated study product instead of smoking if they felt an urge to smoke, and a follow-up phase (weeks 17 to 28). Note that the product was supplied only up to week 16, with subjects instructed to cut down on product use during weeks 14 to 16 to avoid too abrupt an ending of nicotine intake.

The objectives of the US study included comparison of smoking quit rates measured by complete abstention during weeks 6 to 28, verified by CO <8 ppm (the primary objective), comparison of verified quit rates measured by complete abstention during weeks 6 to 16, and comparison of verified quit rates specifically at weeks 16 and 28.

The Serbian study involved a baseline visit, a smoking reduction stage (weeks 1 to 24 post-randomization) and a smoking cessation stage (weeks 25 to 48). During the first 24 weeks subjects were instructed to cut down on smoking by using their allocated product when they felt an urge to smoke, though if they still wished to smoke after 20-30 minutes they could do so. Subjects were also informed that although smoking cessation was preferable, the primary objective of the first 24 weeks of the study was smoking reduction. Subjects who failed to achieve smoking reduction at 24 weeks (as judged by a 50% or more reduction in the self-reported number of cigarettes smoked daily in weeks 20 to 24, and a reduction of at least 1 ppm CO in exhaled air relative to baseline) were not actively followed after the week 24 visit. In all analyses of smoking cessation they were counted as failures. Continuing subjects were instructed to quit smoking completely by use of study products. While the primary objective of the Serbian study related to smoking reduction, other objectives related to smoking cessation, both at weeks 12 and 24, and at weeks 36 and 48.

It should be noted that while snus and placebo were only available to week 16 in the US study, snus and placebo were available to the subjects during the whole period post-

randomization (weeks 1 to 48) in the Serbian study. This difference arose because, while nicotine replacement therapy (NRT) and various smokeless tobacco products are both readily available to all participants at a cost comparable to cigarettes in the USA, smokeless tobacco is not available in Serbia, and the cost of NRT there is considerably more than that of cigarettes.

Data on vital signs (systolic and diastolic blood pressure, body weight, and BMI) were recorded at baseline and during follow up in both studies. Lung function ($FEV_{1,0}$, FVC, FEV%), on the other hand, was only tested among participants in the Serbian trial. The follow-up period in the US trial was considered too short to permit meaningful analyses of changes in lung function. Blood tests for biomarker analyses were taken at baseline and during follow up in both studies. These analyses included: S-WBC, S-CRP, total S-Cholesterol, S-HDL, S-LDL, and S-Fibrinogen. The protocol for the US study included administration of the self-report scale of the Minnesota Nicotine Withdrawal Scale (MNWS) instrument at baseline and during follow up.

Counseling – The protocols for both the Serbian and US trial prescribed counseling of all participants irrespective of allocated treatment. As none of the centers had previous experience with smoking cessation interventions the trialists attended separate training sessions before the trials were initiated. These sessions included lectures on techniques for smoking cessation counseling.

At each clinic and telephone visit in the US trial, subjects were provided with brief counseling by a research nurse that lasted 5-10 minutes, following Agency for Healthcare Research and Quality guidelines (US Pub Health Service, 2011. Agency for healthcare research and quality. Counseling patients to quit. www.ahrq.gov/clinic/tobacco/counsel.htm). In addition, US subjects were provided with an education booklet (the National Cancer Institute's "Cleaning the Air" booklet) prior to randomisation.

In Serbia potential participants were invited to seminars that included information about available smoking cessation strategies. Study subjects were given brief counselling (<5-10 minutes) at each clinical visit by the responsible trialist. According to the protocol the main messages given to participants during the first 24 weeks were the following: *"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 minutes, 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".* Main messages to participants after 24 weeks were: *"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".* The Serbian participants received oral counseling alone.

Statistical analysis – Measurement of abstinence, biochemical verification, and statistical analyses was done according to recommendations by the Society for Research on Nicotine and Tobacco (Hughes, Benowitz, Hatsukami, Mermelstein Shiffman 2004), and methods based on the ICH E9 document "Statistical Principles for Clinical Trials" (European Medicines Agency. Statistical principles for clinical trials 1998, www.ema.europa.eu/pdfs/human/ich/036396en.pdf). Missing responses or missing data related to smoking were interpreted as though the subject had smoked on that occasion. Analyses of efficacy were on the basis of "intention to treat" and no randomized subject was

excluded. Mixed effects repeated measures models were used to assess changes from baseline of e g vital signs and biomarker data in the Serbian trial.

The Statistical Analysis Plans (SAP), which were developed prior to unblinding of the studies, are included in Appendix 1c (Serbia), and Appendix 2b (US)

1.8 Results

Results of the analyses outlined in the SAP for the Serbian trial are enclosed in Appendix 1d. Individual Case Record Form (CRF) data for all participants is enclosed as Appendix 1e. The corresponding data for the US trial are included in the Preliminary Clinical Study Report and Statistical Analysis Plan (Appendix 2b) with corresponding Statistical Outputs (Appendix 2c). Note that the Clinical Study Report for the US trial is only preliminary as it does not include any biomarker data. It is expected that such data will be available in late 2012. Individual Case Record Form data for the US participants are enclosed in Appendix 2d.

The following provides brief summaries of the main findings from the two trials. More detailed information is provided in the published trial reports (Serbian trial: Appendix 1e, US trial: Appendix 2e).

Serbian study - A total of 319 participants entered the study during January, 2008 through April, 2009. The 48-week study completion rates were 56% (88/158) for the snus group, and 63% (101/161) for the placebo group. Among the total of 130 participants who discontinued prematurely, the most common reasons in both treatment groups were failure to achieve the protocol definition of smoking reduction at the week 24 visit (57/130, 43.8%), withdrawal of informed consent (41/130, 31.5%), and loss to follow-up (21/130, 16.2%).

Baseline and demographic characteristics were similar in the snus and placebo groups. Overall, 61% were female. On average, participants were aged 44 years, had smoked 27 cigarettes per day during the past year, and had made 0.6 previous quit attempts. Few participants had previous exposure to nicotine replacement therapy (0.9%) or other pharmaceutical cessation aids (1.3%).

After the first week 97% of participants in both the snus and placebo groups reported some daily use of their allocated study product defined as having used at least one pouch per day during the preceding week. This proportion declined over time and was 52% after 48 weeks in the snus group compared to 60.2% in the placebo group. Among the daily users of snus the mean amount used per day was moderate: the weekly average ranged from 3.5 to 4.7 g per day, but tended to increase over time. Those allocated to the placebo group had a marginally higher consumption.

After the first few weeks c. 70-80% of those who reported daily product use preferred the small, 0.5 g pouches and the mean number of pouches used per day in both treatment groups was c.7-8. This number was similar irrespective of preferred pouch size.

The self-reported mean number of cigarettes smoked per day decreased over time in both the snus group and the placebo group ($p<0.001$). Among those allocated to snus the decrease was slightly, but not statistically significantly more pronounced compared to the placebo group during week 30-48. At the week 48 visit the mean number in the snus group was 7.6 compared to 8.6 in the placebo group.

At the week 24 visit, a total of 101 participants (63.9%) in the snus group achieved smoking reduction according to the protocol definition compared to 109 (67.7%) in the placebo group. This difference was not statistically significant: the estimated odds ratio (snus versus placebo group) was 0.81 (95% C.I.: 0.48-1.36, p=0.42).

Those who did not achieve smoking reduction according to the protocol definition at the week 24 visit were not actively followed during week 24-48. In all analyses of smoking cessation they were counted as failures. This assumption was validated by retrospective telephone interviews which were conducted during December 2009-April 2010. A total of 60 participants had been excluded from active follow-up at the week 24 visit of whom 22 (36.6%%) were contacted and provided information on their smoking history. None of these participants reported having stopped smoking during the week 24-48 period.

Exploratory analyses of the extent of self-reported smoking reduction revealed that more participants in the snus group compared to the placebo group reported 75-100% reductions of their average number of smoked cigarettes per day compared to baseline, particularly during the first six months of the trial. At week 12 this proportion was 9/158 (5.7%) in the snus group versus 3/161 (1.9%) in the placebo group (p=0.07). At week 24 the corresponding proportions were 15/158 (9.5%) and 4/161 (2.5%, p<0.01).

Estimates of CO-verified smoking cessation outcomes are summarized in Table 3. The number of participants with biologically confirmed smoking abstinence during the preceding 4, 12, and 24 week period was higher in the snus group compared to the placebo group at both the week 36 and week 48 visit. The estimated odds ratios ranged from 2.1 to 3.3, but only the estimate for 12-week continued abstinence at week 48 was statistically significant (p<0.05).

The number of participants with CO confirmed 7 day point prevalence abstinence was higher in the snus group compared to the placebo group at the clinical visits week 12, 24, 36, and 48. The estimated odds ratios (snus versus placebo group) ranged from 1.9 to 3.4, but only the estimate at 36 weeks was statistically significant (p<0.05).

Mean blood pressure (systolic and diastolic), body weight, BMI, and the tests for pulmonary function ($FEV_{1.0}$, FVC, FEV %) did not change appreciably over time and there were no statistically significant differences between the two treatment groups.

The levels of S-WBC, S-CRP, total S-Cholesterol, S-HDL, S-LDL, and S-fibrinogen did not change appreciably over time and no statistically significant differences between the treatment groups were observed. In contrast, S-cotinine decreased substantially and similarly over time in both treatment groups (p<0.001): at baseline the mean concentration in the snus and placebo group was 98.9 ng/mL and 101.2 ng/mL, respectively. The corresponding mean concentrations in the two groups during follow up were 70.9 and 70.6 (12 weeks), 68.7 and 71.7 (24 weeks), 62.9 and 69.3 (36 weeks), and 66.1 and 69.1 (48 weeks). Also, CO in exhaled air decreased statistically significantly over time (p<0.001) in both treatment groups: at baseline the mean concentration was 23.5 ppm in both the snus and placebo group. The corresponding mean concentrations in the two groups during follow up were 20.0 and 20.2 (12 weeks), 16.7 and 15.8 (24 weeks), 13.0 and 13.2 (36 weeks), and 11.5 and 12.1 (48 weeks).

The average Fagerström score among those who continued to smoke was lower at the week 24 and 48 clinical visits compared to baseline but there was no difference between participants according to allocated treatment. In the snus group the average score at

baseline, after 24 weeks, and 48 weeks was 6.2, 4.2, and 4.0, respectively. Among the placebo participants the corresponding scores were 6.1, 4.1, and 3.6. The reported decrease in cigarette consumption among participants in both treatment groups contributed to the observed decreases in yhe score as number of cigarettes smoked per day is one out of the six items in the instrument. However, no exploratory analysis of the contribution from the other items was done.

Using snus was safe and generally well tolerated. However, treatment-related adverse events (AEs) were reported more frequently by participants allocated to snus (19.0%) compared to placebo (11.2%), but they were mostly classified as mild and did not result in discontinuation of study treatment. The type of AEs that occurred more frequently in the snus group typically concerned symptoms related to nicotine exposure (tachycardia, nausea, increased salivation, vomiting, and hiccups). Four participants in the snus group (2.5%) were also diagnosed with gingival or buccal irritation compared to one participant (0.6%) from the control group. One participant in the snus group developed a serious AE (severe muscular weakness). It was classified as unrelated to use of study product but led to discontinuation of treatment. Another participant in the snus group discontinued using snus because of an AE (anxiety) which was also classified as unrelated to use of study product. No serious AE was reported among the participants allocated to placebo.

US study - Out of 485 potential participants screened, 250 were included in the trial during February through August, 2009. They were randomly allocated receive either snus or placebo (125 each). One hundred and fifty two participants (61%) completed the study.

Participant characteristics at baseline were comparable between the treatment groups. Overall c. 60% was female. On average, participants were aged c. 45 years, and smoked 20-21 cigarettes per day. About half of the participants reported previous exposure to NRT, and nearly two thirds had tried other pharmaceutical smoking cessation products. The only statistically significant difference was that non-pharmacological smoking cessation aids had been used by 27 participants (21%) allocated to snus compared to 10 participants (8%) in the placebo group ($p<0.05$).

According to the participants' diary data study product usage was relatively limited Participants in the snus group who used 1.0 g sachets consumed on average 3-4 sachets per day. The corresponding number for those who preferred the 0.5 g sachets was 4-8 sachets per day. Those allocated to placebo generally consumed a slightly higher number of sachets per day compared to the snus group, particularly during the first 4-6 weeks of the study. There was no relationship between amount of product use and cessation outcome.

Biologically verified, continuous abstinence during week 6 through 28 was 4.0% and 1.6% for snus and placebo, respectively (Table 4). Statistically significant advantage for snus over placebo occurred for point-prevalence outcomes at weeks 6 and 16. Otherwise the cessation rates were not statistically significantly different between the treatments. For compliant subjects the abstinence rates were one or two percentage points higher than for the "intention to treat" population. The point prevalence rates for snus and placebo were at 6 weeks 21% vs. 10%, $p<.04$; at 16 weeks 19% vs. 9%, $p<.05$ and at 28 weeks 15% vs 8% n.s. (Table 4).

Logistic regression analyses of possible relationships between baseline variables and point prevalence cessation at week 16 indicated that the only predictive variable was low number

of cigarettes smoked per day in the past year. Similar analyses at week 28 suggested that previous use of smokeless tobacco was associated with a higher cessation rate. There were no statistically significant interactions between any of the tested baseline variables and allocated treatment, that is, there was no evidence that the effect of snus was different in any subset of participants.

Snus was generally well tolerated and reported adverse events related to the treatment were mostly classified as mild. A serious AE was reported by two participants in the snus group (pregnancy, vaginal bleeding during pregnancy) none of which was considered related to the allocated treatment, compared to two participants in the placebo group. Five participants in the snus group experienced an AE that led to study discontinuation (sore gums, vaginal bleeding with pregnancy, glossitis & pharyngitis, diarrhea & dyspepsia, and pregnancy), compared to one participant in the placebo group (dysaesthesia). Treatment related AEs more frequently reported in the snus group compared to the placebo group included gingival pain (19% vs 13%), nausea (10% vs 7%), dyspepsia (10% vs 5%), gingivitis (3% vs 1%), salivary hypersecretion (4% vs 0%), dizziness (4% vs 0%), hiccups (6% vs 0%), and pharyngitis (5% vs 2%).

The analyses of MNWS scores for craving showed a decrease over time in both treatment groups that was slightly, but not statistically significantly, greater among those allocated to snus.

The biomarker data from the US study were not available at the time of the publication of the study. They have later been finalized, but as a consequence of the generally low complete quit rates among the participants, comparisons by allocated treatment are generally uninformative. Data are summarized in the appended, complete study report.

1.9 Discussion

The main strength of the two trials was their double-blind, placebo-controlled design, although none of the protocols included procedures to assess the success of the blinding. The main weakness was that the study centers had not previously been involved in smoking cessation programs or worked with either pharmaceutical or behavioral cessation interventions. This may have contributed to the observed relatively low overall quit rate.

Typically, cessation studies including participants motivated to quit report 6 month continuous abstinence rates of 20-30% for active medication and 10-15% for placebo (Silagy et al 2007). Current efficacy results are more comparable to those typically seen in smoking reduction trials including smokers with no immediate wish to stop smoking completely. It is also possible that the negative cultural connotations of using smokeless tobacco in the US, where smokeless tobacco is typically regarded as harmful as cigarette smoking contributed to the observed overall success rates. In Serbia there is no traditional use of any form of oral tobacco products so there are no negative cultural connotations associated with such products. However, the social environment in Serbia with a high smoking prevalence, few smoking restrictions, and a generally low public awareness of the dangers of smoking, is not supportive of quit attempts among smokers who want to stop smoking.

Higher cessation rates with snus are reported in real-life surveys of Swedish and Norwegian smokers (Ramström & Foulds 2006, Lund, McNeill, Scheffels 2010, Lund, Scheffels &

McNeill 2011). This is likely due to self-selection of subjects and perhaps due to phasing in ST use over a much longer period. In the current trials use of study products was relatively limited, although in the Serbian study it tended to increase over time. This suggests that it may take some time before smokers become accustomed to using snus products instead of cigarettes. In the US trial study products were not available to participants after week 16.

Beneficial effects from smoking reduction or cessation on vital signs (e.g. blood pressure and pulmonary function) and biomarker levels (e.g. CRP, fibrinogen, and blood lipids) are mainly observed among those who quit completely and typically take several weeks to months to emerge. The overall low complete cessation rates in the Serbian study may have contributed to the fact that no statistically significant overall differences between the treatment groups were observed in terms of such measures, despite the difference in number of quitters in favor of the snus group. Any differences that may have occurred were probably obscured by the results for the large number of non-quitters. The generally small number of quitters also precluded meaningful exploratory analyses based on subsets according to quitting behavior.

In summary, both the US and Serbian trials showed that biologically verified cessation rates were consistently 2-3 times higher among subjects allocated to snus although small numbers in both studies contributed to a low statistical precision of the effect estimates as well as in analyses of treatment interactions.

Systematic review and meta-analysis

2.1 Abstract

A systematic review and meta-analysis was conducted of randomized clinical trials of Swedish snus that included long term smoking cessation as an end point. It was evident that the Serbian and US clinical trials were the only such trials.

The rationale for a joint analysis of the two studies was that a formal meta-analysis of appropriately defined end-points would improve statistical precision and allow better insight into the main hypothesis of interest, that is, those related to biologically verified, complete smoking cessation. Although there were differences in the design of the two trials, they had enough similarities (e g both were randomized, double-blind, placebo-controlled trials testing whether *ad lib* provision of snus affected subsequent smoking habits among adult smokers) to make it worthwhile to combine the evidence from the two studies to allow more powerful tests of whether use of snus affects the rate of quitting smoking. Given that meta-analyses are frequently conducted for observational epidemiological studies, where there may be variation in study design, type of exposure, and extent of adjustment for potential confounding variables, there can be little objection to meta-analysis of relatively similar randomized controlled trials with the same active and placebo treatments.

Both studies were of cigarette smokers of a similar age range and similar minimum daily cigarette consumption who did not use smokeless tobacco. The US study involved a 4 week post-randomization period during which the subjects were instructed to use the study products when they felt an urge to smoke, initially without any requirement for complete abstention, a 12 week intervention phase during which subjects were encouraged to quit smoking completely and to use their allocated study product if they felt an urge to smoke, and a follow-up phase of 12 weeks. The Serbian study involved a 24 week post-randomization period during which they subjects were instructed to use the study products to cut down or preferably quit when they felt an urge to smoke, and a 24 week smoking cessation period, during which the subjects were instructed to quit smoking completely by use of the study products.

The primary outcome in the meta-analysis was continuous smoking cessation (at each of weeks 6 to 28 US and 24 to 48 Serbia, confirmed by exhaled air CO values (<8 ppm at weeks 6, 10, 16 and 28 US and <10 ppm at weeks 24, 36 and 48 Serbia). Five secondary outcomes were defined based on continuous smoking cessation (CO confirmed) for shorter periods or based on being smoke-free (CO confirmed) at specific weeks. Analyses were primarily conducted using the intention-to-treat population. This was defined as all eligible subjects who had a baseline evaluation, and were randomized to receive one of the study products, irrespective of compliance and protocol violations. Some additional analyses were conducted restricted to compliant subjects, based upon the definition used for the Serbian study. For that study, this required the subject to use the study product on each day during weeks 1 to 24. For the US study, the definition used required that the subject used the study product on average at least once a day in each of weeks 1 to 5. These time periods represented “grace periods” before the advice to the participants focused on complete smoking cessation.

Seven baseline characteristics were defined that might possibly be related to the outcomes; gender, age at entry, average number of cigarettes smoked per day in the year before baseline, age at starting to smoke, baseline Fagerström nicotine dependence score, whether

or not previously attempted to quit smoking, and whether or not previously used pharmaceutical nicotine.

A preliminary analysis presented the distributions of these seven baseline characteristics within study and overall, and compared the two studies using exact tests for variables with two levels (gender, previous quit attempt, previous use of pharmaceutical nicotine) and Wilcoxon rank tests for the remaining, continuous, variables.

The distribution of the seven baseline characteristics was compared between the active and placebo groups, as a test of failure of randomization. Comparisons were made within study and overall (adjusted for study). For the adjusted ("stratified") analysis, stratified chi-squared tests and stratified rank tests were used.

For each of the six defined cessation outcomes, success rates were compared by level of each of the seven baseline characteristics, within study and overall (adjusted for study). For the continuous variables, levels were defined to include approximately equal number of subjects, and the analysis included a test for trend.

For each of the defined outcomes, success rates were then compared by treatment, within study and overall (adjusted for study). Statistical tests of treatment effects were conducted using exact tests, with the relative risk for active to placebo, and its 95% confidence interval, estimated by methods appropriate for fixed-effect models based on the logit method. Tests for heterogeneity of the relative risk over study were conducted, but were never significant ($p<0.05$), so random-effects meta-analysis was not attempted.

Corresponding analyses were also carried out stratified for study site rather than study, and also, for each baseline characteristic, stratified both by study and by two levels of the characteristic, the levels being chosen to divide the population into two approximately equal groups.

In all analyses, p-values <0.05 (two-sided) were considered statistically significant. It should be noted that, as the p-values were based on exact tests, and the estimates of relative risk and confidence intervals on approximate, asymptotic, tests, it was possible for the 95% confidence interval not to include 1.0 when the exact p-value was >0.05.

There were 250 subjects (125 active, 125 placebo) in the US study, and 319 (158 active, 161 placebo) in the Serbian study. The studies had a similar frequency of males, and the subjects were of similar age and age of starting to smoke. Subjects in Serbia had higher mean average daily cigarette consumption at baseline, 26.7, than subjects in the USA, 20.4, and a higher Fagerström nicotine dependence score. They were also less likely to have made a previous quit attempt, and very much less likely to have used NRT. There was no evidence in either study of failures of randomization, with the distribution of sex, age, cigarette consumption, age of starting to smoke, nicotine dependence score, previous quit attempts and previous use of NRT similar in the active and placebo groups.

None of the outcomes were significantly ($p<0.05$) related to sex, age, age of starting to smoke, nicotine dependence score, previous quit attempts or previous use of NRT. However, in the studies combined, there was a significant ($p<0.01$) tendency for overall outcome success rates to be greater in lighter smokers (10-19 cigarettes/day) compared to those smoking 20+ cigarettes/day.

Only seven subjects in the USA and 12 in Serbia were successes as defined by the primary outcome. Although the relative success rate exceeded 2-2.5 in both the US (2.50, 95% CI 0.49-12.65) and Serbia (3.06, 0.84-11.08), the meta-analysis estimate (2.83, 1.03-7.75) was of borderline statistical significance (exact p: 0.06, chi-squared p: 0.03). Adjustment for previous cigarette consumption reduced the estimate to 2.10 (0.77-5.76), but the exact p value remained at 0.06. There was no evidence ($p>0.6$) that the relative success rate for any of the defined outcomes was different in the two studies.

Although estimated success rates with snus were higher in smokers reporting an average baseline consumption of 20+ cigarettes per day (RR 6.52, 95% CI 1.18-36.15) than in smokers of 10-19 cigarettes per day (RR 1.15, 0.33-4.01), this heterogeneity was not statistically significant ($p=0.10$). There was no statistically significant evidence that the success rate with snus was related to any of the other studied baseline variables, although small numbers contributed to low statistical precision.

For the combined data, success rates for the five secondary outcomes were typically about twice as high in the active group as in the placebo group. For all five outcomes, this excess was statistically significant ($p<0.05$).

The significant excess among those allocated to snus for smoking cessation (CO confirmed) in the last four weeks of the study period, with success rates of 12.4% for snus vs. 6.6% for placebo (RR 1.86, 95% CI 1.09-3.18), suggests that there is a real and clinically worthwhile advantage to snus in encouraging quitting.

2.2 Introduction & rationale

It is clear that the US and Serbian trials are the only controlled trials of Swedish snus or snus-type smokeless products including long term smoking cessation as an end-point. The lack of such data was noted by an EU-commissioned report in 2008 (SCENIHR 2008), was reiterated by several Nordic public health officials in 2009 (Holm L-E, Fisker J, Larsen B-I, Puska P, Halldórsson M 2009), and was confirmed by negative findings of searches in MedLine and clinical trial data bases. A meta-analysis of the US and Serbian trials may therefore be regarded as a systematic review of all the available data.

In 2006 Tönnesen et al published the results of a smoking cessation trial testing a Danish smokeless tobacco product (Tönnesen et al 2006). However, aside from being smokeless, that product has little resemblance to snus. It is classified in the EU as chewing tobacco and has not been characterized in the literature in terms of e g product chemistry or nicotine delivery. Consequently it was not included in the current meta-analysis.

In each of the two trials the target sample size was originally determined so that the studies would have >80% statistical power to detect ($p<0.05$) a slightly more than two-fold increase of the success rate among those allocated to snus in terms of the primary end-point. These considerations resulted in target sample sizes that, although moderate, are frequently seen in smoking cessation trials (312 for the Serbian trial, and 250 for the US trial). The mentioned type of power calculations is dependent on the accuracy of the estimates of the outcome in the control group. The ability of snus to promote smoking cessation had previously not been tested in controlled settings, and the participating centers had no previous experience with clinical smoking cessation interventions. Therefore, it was clear already before the trials were initiated that sample size and statistical precision might be an issue in analyses of cessation outcomes as the defined number of participants in both studies was limited.

Although there were differences in the design of the two trials, they had enough similarities (e g both were randomized, double-blind, placebo-controlled trials testing whether *ad lib* provision of snus affected subsequent smoking habits among adult smokers) to make it worthwhile to combine the evidence from the two studies to allow more powerful tests of whether use of snus affects the rate of quitting smoking. It was considered reasonable to assume that a formal meta-analysis of appropriately defined end-points would improve statistical precision and allow better insight into the main hypothesis of interest. No attempt was made to jointly analyze data on end-points other than those related to biologically verified, complete smoking cessation.

The systematic review and meta-analysis was done by an external contractor (P N Lee Statistics and Computing Ltd, PNLSC). It was done according to a pre-specified protocol and statistical analysis plan (Appendix 3a), was based on individual participant data for each of the two studies, and conformed to the internationally accepted PRISMA guidelines (http://www.prisma-statement.org). The data were transferred to the contractor in SAS-format from the CROs responsible for data analyses in the two trials (i3Staprobe, Covance). A comprehensive study report including the individual patient data used in the meta-analysis, as well as a scientific paper (submitted) summarizing the main findings are enclosed as Appendix 3b and Appendix 3c.

2.3 Definition of smoking cessation end-points

In deriving endpoints for the meta-analysis, it seemed appropriate to consider the period following when the advice given to subjects concentrated on quitting, i.e. from week five in the US study and from week 25 in the Serbian study. To avoid bias, it was also appropriate to base the main analyses on all the subjects randomized initially, which implied including those not actively followed after week 24 in the Serbian study because they failed to achieve a ≥50% reduction in the number of cigarettes smoked daily, and consequently were assumed not to be going to quit had they continued in the study.

The primary objective of the meta-analysis was to examine if snus compared to placebo, increased the quit rate among the study participants, as quantified by continuous, complete smoking cessation over an approximate 23-24-week period following advice to quit smoking. For the US study, this was defined as complete abstention during weeks 6 to 28 verified by expired air CO less than 8 ppm at all clinical visits. For the Serbian study, this was defined as complete abstention during weeks 24 to 48, verified by expired air CO less than 10 ppm at all clinical visits.

The secondary objectives were:

- To examine point-prevalence (preceding week) quit rates (biologically confirmed) at the following defined pairs of times in the two studies;

  weeks 6 US and week 24 Serbia,

  week 16 US and week 36 Serbia,

  week 28 US and week 48 Serbia.

- To examine the prevalence of smoke-free subjects (self-reported and confirmed by CO measurement) at weeks 25-28 in the US study and at weeks 45-48 in the Serbian study.

- To examine the prevalence of smoke-free subjects (self-reported and confirmed by CO measurement) at weeks 17-28 in the US study and at weeks 37-48 in the Serbian study.

A graphical description of the defined meta-analysis end points is shown in Figure 3.

2.4 Data

The contractor (PNLSC) was supplied by the sponsor (SM) with electronic data files (in SAS format) for each of the two studies containing information for each subject randomized. This information included:

- Site where the subject attended
- Sex
- Age
- Smoking history
- History of quit attempts and use of cessation aids
- Date at which the subject was randomized
- Whether the subject was randomized to snus or placebo
- Results of the CO Exhaled air test (weeks 0, 6, 10, 16 and 28 for the US study; weeks 0, 2, 6, 12, 18, 24, 30, 36, 42 and 48 for the Serbian study)

- Results of the Fagerström test conducted at baseline
- Self-reported smoking status for each week of the study
- Responsible trialists' assessment of defined smoking cessation end-points based on participant's diary data and CO tests
- Whether the subject used the study product (snus or placebo) for each week of the study
- Whether the subject completed the study or was withdrawn
- Whether the subject reduced or stopped smoking (Serbian study only, weeks 12, 24, 36 and 48)
- Date of completion or withdrawal

Populations **-** Analysis was mainly carried out using the intention-to-treat population. This was defined as all eligible subjects who had a baseline evaluation, were randomized to receive one of the study products, irrespective of compliance and protocol violations.

Some additional analyses were conducted based on compliant subjects, defined as those who used the product on average at least one day a week in each of weeks 1 to 5 (US) or weeks 1 to 24 (Serbia).

Data processing - The relevant individual subject data were transferred onto two similarly structured and linked databases, one for each study, to allow for statistical analysis.

The distributions of the variables used in the analysis were compared with output prepared by the external contractors responsible for all statistical analyses of the individual trial data) to ensure that the transfer had been successful.

Outcomes - For the US study the primary outcome measure was continuous smoking cessation during weeks 6 to 28 inclusive based on the trialist's assessment of diary readings recorded at these weeks confirmed by exhaled air CO values of less than 8 ppm at weeks 6, 10, 16 and 28.

For the Serbian study the primary outcome measure was continuous smoking cessation during weeks 24 to 48 inclusive based on the trialist's assessment of diary readings recorded at those weeks confirmed by CO values from exhaled air of less than 10 ppm at weeks 24, 36 and 48.

Secondary outcome measures were:

1. The point prevalence of smoke-free subjects (confirmed by CO measurement) at week 6 in the US study and at week 24 in the Serbian study.

2. The point prevalence of smoke-free subjects (confirmed by CO measurement) at week 16 in the US study and at week 36 in the Serbian study.

3. The point prevalence of smoke-free subjects (confirmed by CO measurement) at week 28 in the US study and at week 48 in the Serbian study.

4. Continuous smoking cessation (confirmed by CO measurement) at weeks 25-28 in the US study and at weeks 45-48 in the Serbian study.

5. Continuous smoking cessation (confirmed by CO measurement) at weeks 17-28 in the US study and at weeks 37-48 in the Serbian study.

Missing values for smoking status or CO measurement were taken as indicating that the subject smoked on that occasion. The diary readings used had been reviewed by the responsible trialist at weeks 6, 10, 16 and 28 in the US study, and at weeks 24, 36 and 48 in the Serbian study.

2.5 Statistical methods

Variables other than study treatment (active or placebo) considered as potential confounding variables were the following:

- Site where the subject attended (five in the USA, two in Serbia)
- Sex
- Age
- Average cigarettes smoked per day in the year before baseline
- Age at starting to smoke
- Fagerström nicotine dependence score at baseline
- Previous quit attempt
- Previous use of NRT

Statistical analyses test for variation in a response by levels of a factor both within each level of a partitioning variable, and also overall, with adjustment for the partitioning variables.

The analyses can be divided into five types:

	Response	Factor	Partitioning variable
1.	Outcome	Potential confounding variable	Study
2.	Potential confounding variable	Study product	Study
3.	Outcome	Study product	Study
4.	Outcome	Study product	Study site
5.	Outcome	Study product	Study, potential confounding variable

Analyses of type 1 give background information on variables other than the study product that might affect outcome. For some variables, e.g. sex, the factor only has two levels.

However, for continuous or semi-continuous variables, the factor was categorized to include up to five monotonically increasing levels, based on inspection of the distribution of the variable so as to have approximately equal number of subjects in each category. In this case the analysis included a test for trend, i.e. for response to increase or decrease with successive levels of the factor variable.

Analysis of type 2 investigates possible failures of randomization. Variables showing differences between snus and placebo that are significant at $p<0.05$ were considered as partitioning variables in the type 5 analyses, and if none were significant, the analysis was not carried out.

Analyses of types 3 to 5 (the meta-analyses) test for a relationship between study product and outcome, and concern the main hypothesis of interest – the effect of snus on the quit rate. The analyses presented are fixed-effect meta-analyses based on the logit method (Fleiss & Gross 1991). Alternative analyses using the Mantel-Haenszel method (Greenland & Robbins 1985) gave very similar results (data not shown).

The intention was originally to also conduct random-effect meta-analyses where there was significant ($p<0.05$) evidence of heterogeneity of the relative risk over partitions, but as this never occurred, only the fixed-effect methods are described below.

For the meta-analyses, the data for each level of the partitioning variable (i = 1, 2 … s) can be laid out as follows:

Subjects	Active (Snus)	Placebo
Outcome successful (quit smoking)	A_i	B_i
At risk	C_i	D_i

The relative risk for level i is estimated by

$RR_i = (A_iD_i)/(B_iC_i)$

and the variance of log RR_i, V_i, is estimated by (Katz et al 1978),

$V_i = \text{var}(\log_e RR_i) = 1/A_i + 1/B_i - 1/C_i - 1/D_i$

The combined fixed-effect estimate of $\log_e RR$ over the partitions is calculated as an inverse-variance weighted average of the individual estimates. Thus, we have

$$Y_T = \log_e RR_T = \left(\sum_{i=1}^{s} w_i \log_e RR_i\right) / \sum_{i=1}^{s} w_i$$

where w_i, the weight, equals $1/V_i$.

Thus the overall estimate RR_i is given by exp (Y_T).

The variance of $\log_e RR_T$, V_T, is then estimated by

$$V_T = \text{var}(\log_e RR_T) = 1 / \sum_{i=1}^{s} w_i$$

A 95% confidence interval for the overall estimate of RR_T is then given by

exp ($Y_T \pm Z\sqrt{V_T}$)

Where Z is the value of the standard normal distribution corresponding to the 97.5% percentile (i.e. approximately 1.96).

The output from the analyses also includes the results of unstratified and stratified chi-squared and exact tests. Inference was always based upon the exact probabilities where these could be calculated. The chi-squared tests were not Yates' corrected as then the resulting chi-squared statistics and probabilities relate directly to the relative risks. It should be noted that due to the small number of cases there was appreciable differences between the approximate asymptotic probabilities and the exact probabilities. The results of a test of the heterogeneity of the relative risk over the partitions is also shown, based on taking the statistic

$$Q = \sum_{i=1}^{s} w_i (Y_i - Y_T)^2$$

as having a chi-squared distribution with s-1 degrees of freedom (Fleiss & Gross 1991).

It should be noted that, as p-values are based on exact tests, and the estimates of relative risk and confidence intervals are based on approximate asymptotic tests, it is possible for the 95% confidence interval not to include unity when the exact p-value is >0.05. Therefore, for the meta-analysis both exact and chi-squared p-values are given to avoid any ambiguity.

The methods used for the analyses of type 1 are the same as for the meta-analyses, except that the basic data table is changed to:

	Potential confounding variables			
Subjects	Level 1	Level 2	….	Level s
Outcome successful (quit smoking)	A_1	A_2		A_s
At risk	C_1	C_2		C_s

with relative risks expressed compared to a reference level of the confounding variable. Here the analyses also include tests for overall variation between groups and for trend (Breslow & Day 1980).

For analyses of type 2, where the potential confounding variable has two levels, the methodology was similar to that for the analyses of types 3, 4 and 5, except that the basic data table is changed to, e.g.

Subjects	Snus	Placebo
Males	A_1	B_1
Total	C_i	D_i

and the statistic $(A_iD_i)/(B_iC_i)$ measures the relative frequency of males in the two groups.

For analyses of type 2, where the potential confounding variable is continuous or semi-continuous, comparisons of snus and placebo were carried out using Wilcoxon rank tests (Conover 2003) to compare the median response in the two groups. Other descriptive statistics are also presented, such as the mean and standard deviation. For an overall comparison over levels of the partitioning variable, a stratified version of the Wilcoxon rank test was used (Fry & Lee 1991).

In the above analyses of continuous or semi-continuous potential confounding variables the following levels were used as factors, partitioning variables or trend coefficients:

	Levels				
Potential confounding variable	1	2	3	4	5
Age	20-36 (base)	37-45	46-52	53-64	-
Average cigarettes smoked per day in the year before baseline	10-19 (base)	20	21-30	31-60	-
Age of starting to smoke	8-15 (base)	16-17	18-19	20-21	22-53
Baseline Fagerström score	0-4 (base)	5	6	7	8-10

2.7 Results

A full report of the meta-analysis including individual subject data is enclosed as Appendix 3b. A paper summarizing the meta-analysis has been published (Rutqvist, Fry Lee, 2013). The following text provides a brief description of the main findings.

Baseline variables - Table 5 shows the distribution of the potential confounding variables (sex, age, cigarettes per day, age of starting to smoke, Fagerström dependency test score, previous quit attempts and previous use of NRT) by study. No significant ($p<0.05$) differences between study were seen in sex, age or age of starting to smoke. However, significant ($p<0.001$) differences were seen in the other four variables, with subjects in Serbia having a higher average cigarette consumption per day, a higher Fagerström nicotine dependence score, and a lower likelihood of having made a previous quit attempt or having previous exposure to NRT.

Variation in outcome for other major variables than treatment - There was no significant evidence that any outcome varied by sex in either study, or overall. Nor was there any significant evidence of a tendency for any outcome to show an increasing or decreasing trend in relation to age, age of starting to smoke or Fagerström dependency score. However, as shown in Table 6, there was a clear tendency for all the outcomes to show a

reducing trend in relation to increasing daily cigarette consumption. The trend was significant ($p<0.05$) for all the outcomes for the overall data and for Serbia, but only significant for secondary outcomes 1 and 2 for the USA. This may partly reflect the smaller number of heavier smokers in the US study. Relative success rates in smokers of 10-19 cigarettes per day compared to smokers of 31-60 cigarettes per day ranged from over 2.5 to about 7.

Testing for possible effects of randomization - Based on the results in Table 7, there was no evidence for either study, or overall, of any failure of randomization, with the proportion who were male, who had made a previous quit attempt, and who previously used NRT, and the mean age, cigarette consumption in the year before baseline, age started smoking and Fagerström dependency score similar in the active and placebo groups. T

Effects of treatment- In the two studies combined, only 19 subjects were successes as defined by the primary outcome, 7 in US and 12 in Serbia. Although the success rate was higher in the active group in both studies, with the relative risk estimate exceeding 2-2.5 in both the US (2.50, 0.49-12.65) and Serbia (3.06, 0.84-11.08), the meta-analysis estimate (2.83, 1.03-7.75) was of borderline statistical significance (exact p: 0.06, chi-squared p: 0.03) (Table 8).

All the secondary outcomes showed an advantage to the active group in both studies (Table 8). Because they involved more total successful outcomes than was the case for the primary outcome, many of the relative risk estimates are statistically significant at $p<0.05$. Indeed, for the results meta-analyzed over study, all five estimates are about 2.0 and statistically significant.

For all defined outcomes, there was no evidence ($p>0.6$) of heterogeneity of the relative risk estimates by study. As a result (data not shown), random-effects estimates of the overall relative risk over the two studies were identical to those shown in Tables 8.

The individual trial and meta-analysis results for the primary and all secondary outcomes are displayed graphically in Figures 4-9.

The overall relative risk estimates from analyses adjusted for study site were somewhat lower than those shown in Table 8: primary outcome 2.36 (0.92-6.05); secondary outcomes 1 to 5 in turn 2.02 (1.12-3.64), 2.11 (1.21-3.70), 1.66 (1.03-2.67), 1.68 (0.98-2.87) and 1.84 (0.95-3.56). However, the conclusion that the increased success rate in the active group was borderline statistically significant for the primary outcome, but clearly significant for all of the secondary outcomes, remained the same.

Due to the significant difference in the outcomes by smoking intensity, it was decided to examine whether the estimated effect of treatment on outcome changed when this cofactor was allowed for. As the studies varied in smoking intensity the results were also stratified for study. The resulting relative risks were somewhat lower though the significance of the effect of treatment was fairly similar for each outcome, with the relative risk for the primary outcome now estimated at 2.10 (0.77-5.76) (exact p: 0.06).

Treatment interactions - While the estimated effect of treatment was always greater for smokers of 20+ cigarettes/day than for smokers of 10-19 cigarettes/day, significant heterogeneity was only evident for two of the secondary outcomes (cessation at week 6 in USA and 24 in Serbia, $p = 0.02$; cessation at week 16 in USA and 36 in Serbia, $p = 0.02$), the p-value being ≥ 0.1 for the other four outcomes). For the primary outcome the RR was 1.15

(0.33-4.01) for smokers of 10-19 cigs/day, where 5 of 9 quitters were in the active group, and 6.52 (1.18-36.15) for smokers of 20+ cigs/day, where 9 of 10 quitters were in the active group, with the heterogeneity p = 0.104. There was no statistically significant evidence that the effect of treatment varied according to any of the other studies baseline variables.

Compliant subjects - Analyses were done restricted to compliant subjects, defined as those who used the product on average at least one day a week in each of weeks 1 to 5 (US) or weeks 1 to 24 (Serbia). Compared to the intention-to-treat population, which included 250 subjects in the US (125 active, 125 placebo) and 319 in Serbia (158 active, 161 placebo), these analyses were based on 200 subjects in the US (99 active, 101 placebo) and 255 in Serbia (122 active, 133 placebo), with no significant evidence in either study that non-compliance rates varied by treatment.

The combined relative risk estimates (Table 9) were quite similar to those in Table 8 with a significant ($p<0.05$) advantage to the active treatment again seen for secondary outcomes 1-5. For the primary outcome, the estimate, now based on 16 successful outcomes rather than 17, was 3.09 (1.00-9.55, exact p: 0.054).

2.10 Discussion

The US and Serbian studies are to some extent different, partly because of the ready availability of smokeless tobacco products and pharmaceutical smoking cessation aids in the US, but not in Serbia, and the vastly different social contexts in terms of smoking habits and attitudes to smoking in the US compared to Eastern Europe. However, the designs of the studies are similar enough to allow valid meta-analysis. Given that meta-analyses are frequently conducted for observational epidemiological studies, where there may be variation in study design (e.g. case-control or prospective cohort), type of exposure, and extent of adjustment for potential confounding variables, there can be little objection to meta-analysis of relatively similar randomized controlled trials with the same active and placebo treatments.

A major strength of this meta-analysis was that it was based on individual subject data which allowed comparable definitions of outcomes and potential confounding variables, identical statistical analyses to be conducted for the two studies, and the calculation of exact rather than approximate probabilities for the statistical tests.

In the main analysis, based on the primary outcome (biochemically validated continuous smoking cessation during 23-24 weeks) for the intention to treat population, there was an increased success rate in the group allocated to receive snus. However this was based on only 14 successes (4.9% success rate) in the snus group, as against 5 (1.7% success rate) in the placebo group, and the relative success rate of 2.83 was borderline significant (exact p: 0.06, chi-squared p: 0.03), with the 95% confidence interval 1.03-7.75. However, this outcome represents quite a stringent criterion, with the subjects having not to smoke at all over a period of several months. Success rates were substantially higher, and the advantage to snus generally statistically significant for criteria based on success at specific weeks, or based on shorter periods. Notably this was true for criteria involving the end of the follow-up period, including secondary outcome 3 (smoke-free at week 28 in the USA and at week 48 in Serbia, with an RR of 1.73, 95% CI 1.07-2.78, based on combined study rates of 14.5% for snus and 8.4% for placebo) and secondary outcome 4 (cessation at weeks 25-28 in the USA and at weeks 45-48 in Serbia, with an RR of 1.86, 95% CI 1.09-3.18, based on combined study rates of 12.4% for snus and 6.6% for placebo). This strongly suggests that there is a

real advantage to snus in encouraging quitting. It should be pointed out, however, that there is no information from the trials on whether those subjects who were smoke-free at the end of the study period were still smoke-free 6 months or a year later, and whether those who were, continued to use smokeless tobacco or were tobacco-free. Epidemiological evidence from Sweden indicate that a substantial proportion of those who quit smoking by switching to snus continue to snus long term.

In the studies combined, there was a statistically significant tendency for outcome success rates to be higher in lighter smokers which confirms and extends previous information from other cessation studies. None of the outcomes were significantly related to gender, age, age of starting to smoke, nicotine dependence score, previous quit attempts, or exposure to pharmaceutical nicotine. However, the small number of observations limited the statistical power to detect heterogeneity.

A recent Cochrane overview of placebo-controlled, randomized NRT trials showed that long term quit rates increased about 1.8-fold among those allocated to active treatment (Silagy 2007). The current results for snus reported appear to be at least on par with or slightly above that level of efficacy. Such a conclusion is consistent with data from clinical studies on nicotine uptake from Swedish snus compared with nicotine chewing gum which show that the uptake from snus is comparable to but generally faster than from commercially available nicotine gum (Lunell & Lunell 2005, Lunell & Curvall 2011). Also, a randomized clinical trial including 63 smokers based on an open-label, crossover design showed that during a two week test period Swedish snus was superior to a 4 mg nicotine gum in terms of reducing urges to smoke compared to baseline, although the decrease in total craving score was not statistically significant for either product (Caldwell et al 2010). Compared with baseline there were comparable reductions with both products in terms of the craving subscale of the Minnesota Nicotine Withdrawal Scale. The two products enabled subjects to reduce their smoking significantly compared to baseline ($p<0.01$). At the end of the test period participants were asked to rank their preferred purpose for using the products if they could use them long term. Subjects could choose from three uses: “short term to quit smoking”, “to reduce smoking”, and “long term instead of smoking”. Snus was ranked higher than the gum in all three dimensions and the difference was statistically significant for the “quit” and “reduce” dimensions.

In summary, the joint analysis of the US and Serbian trial provides experimental evidence on the efficacy of Swedish snus to promote long-term, complete smoking cessation among adult, daily smokers motivated quit or substantially reduce their smoking. This evidence concurs with population data from Scandinavia which show that many smokers have used snus as a quitting aid. There was some evidence that the effect of snus was greater among heavier smokers defined as those reporting smoking 20+ cigarettes per day at baseline compared to those who smoked 10-19 cigarettes per day. There was no statistically significant evidence that effects of snus differed according to any of the other studied baseline variables. However, small numbers limited the power to detect significant heterogeneity.

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Tables:

Figures:

Table 1: Participant disposition according to Fagerström score in the Serbian clinical feasibility study

Serbian clinical feasibility study: participant disposition according to Fagerström score

	Snus	Nicotine patch
	Number of subjects	
Moderate smokers (FNTD <6)		
Males	5	1
Females	3	3
Heavy smokers (FNTD ≥6)		
Males	10	12
Females	3	2
Total:		
Males	15	13
Females	6	5
M+F	21	18

Table 2: Results of the Serbian clinical feasibility study in terms of average CO values in exhaled air at baseline and at the end of the 1 month follow-up period

Serbian clinical feasibility study: results

	Allocated snus		Allocated nicotine patch	
	Average CO-level (ppm):			
	Baseline	After 1 month	Baseline	After 1 month
Moderate smokers:				
Males	6.0	5.0[1]	6.0	0
Females	15.5	9.0	22.5	4.5[2]
All	10.7	7.0	14.2	4.5
Heavy smokers:				
Males	35.0	4.5	21.1	10.0[3]
Females	20.0	15.3	39.5	5.0
All	27.5	9.9	23.7	9.3
All subjects (range)	27.2 (5-59)	7.8 (3-25)	27.3 (5-40)	8.0 (3-24)

1: Including 1 subject who stopped smoking
2: Including 3 subjects who stopped smoking
3: Including 1 subject who stopped smoking

Table 3: CO-verified smoking cessation outcomes in the Serbian trial

Outcome	Snus, n=158 (%)	Placebo, n=161 (%)	Odds ratio (snus vs placebo)	95 % C.I.	P
Continued cessation at week 36:					
-4 weeks	13 (8.2)	6 (3.7)	2.3	0.9-6.4	0.10
-12 weeks	9 (5.7)	3 (1.9)	3.3	0.9-12.5	0.08
-24 weeks	2 (1.3)	0	-	-	-
Continued cessation at week 48:					
-4 weeks	22 (13.9)	12 (7.5)	2.1	1.0-4.4	0.06
-12 weeks	15 (9.5)	6 (3.7)	2.7	1.0-7.3	0.04
-24 weeks	9 (5.7)	3 (1.9)	3.3	0.9-12.5	0.08
Point-prevalence cessation (1 week):					
-week 12	2 (1.3)	0	-	-	-
-week 24	9 (5.7)	3 (1.9)	3.4	0.9-12.8	0.08
-week 36	15 (9.5)	6 (3.7)	2.7	1.0-7.3	0.04
-week 48	25 (15.8)	15 (9.3)	1.9	0.9-3.7	0.08

Table 4: CO-verified smoking cessation outcomes in the US trial

	Snus, n=125 (%)	Placebo, n=125 (%)	Odds ratio (snus vs placebo)	95 % C.I.	P
Continuous cessation:					
-week 6-16	9 (7.2)	4 (3.2)	2.3	0.7-7.8	0.15
-week 6-28	5 (4.0)	2 (1.6)	2.5	0.4-13	0.45
Point prevalence cessation (1-week):					
-week 6	23 (18.4)	11 (8.8)	2.3	1.0-5.0	0.03
-week 16	22 (17.6)	10 (8.0)	2.4	1.1-5.4	0.02
-week 28	16 (12.8)	9 (7.2)	1.9	0.8-4.4	0.14

Table 5: Distribution of baseline variables in the US and Serbian trial[a]

Variable	Level	US	Serbia	Signif[b]	Combined
Sex	Male	98 (39.2%)	123 (38.6%)		221 (38.8%)
	Female	152 (60.8%)	196 (61.4%)	NS	348 (61.2%)
Age	20-36	65 (26.0%)	82 (25.7%)		147 (25.8%)
	37-45	54 (21.6%)	84 (26.3%)		138 (24.3%)
	46-52	62 (24.8%)	84 (26.3%)		146 (25.7%)
	53-64	69 (27.6%)	69 (21.6%)		138 (24.3%)
	Mean	45.04	43.64	NS	44.25
Average cigarettes smoked per day in the year before baseline	10-19	84 (33.6%)	32 (10.0%)		116 (20.4%)
	20	108 (43.2%)	114 (35.7%)		222 (39.0%)
	21-30	45 (18.0%)	112 (35.1%)		157 (27.6%)
	31-60	13 (5.2%)	61 (19.1%)		74 (13.0%)
	Mean	20.40	26.66	+++	23.91
Age of starting to smoke	8-15	77 (30.8%)	54 (16.9%)		131 (23.0%)
	16-17	58 (23.2%)	67 (21.0%)		125 (22.0%)
	18-19	44 (17.6%)	89 (27.9%)		133 (23.4%)
	20-21	33 (13.2%)	59 (18.5%)		92 (16.2%)
	22-53	38 (15.2%)	50 (15.7%)		88 (15.5%)
	Mean	18.39	19.01	NS	18.74
Fagerström nicotine dependence score at baseline	0-4	72 (28.8%)	68 (21.3%)		140 (24.6%)
	5	49 (19.6%)	41 (12.9%)		90 (15.8%)
	6	48 (19.2%)	60 (18.8%)		108 (19.0%)
	7	35 (14.0%)	58 (18.2%)		93 (16.3%)
	8-10	46 (18.4%)	92 (28.8%)		138 (24.3%)
	Mean	5.55	6.17	+++	5.90
Previous quit attempt	Yes	219 (87.6%)	116 (36.4%)		335 (58.9%)
	No	31 (12.4%)	203 (63.6%)	−−−	234 (41.1%)

Previous NRT exposure	Yes	126 (50.4%)	3 (0.9%)	−−−	129 (22.7%)
	No	124 (49.6%)	316 (99.1%)		440 (77.3%)
Number of subjects		250	319		569

[a] The table shows the number of subjects, and the percentage of the study population

[b] NS $p \geq 0.05$; +++ $p<0.001$ Serbia > US; --- $p<0.001$ USA < Serbia.

Table 6: Meta-analysis based on the US and Serbian trials of the relationship of outcome to average daily cigarette consumption in the year before baseline[a]

		US	Serbia	Total
Outcome	**Cigs/day**	**n (%)**	**n (%)**	**n (%)**
Primary outcome:				
Cessation weeks 6-28 (US)	10-19	4 (4.8%)	5 (15.6%)	9 (7.8%)
weeks 24-48 (Serbia)	20	3 (2.8%)	4 (3.5%)	7 (3.2%)
	21-30	0 (0%)	2 (1.8%)	2 (1.3%)
	31-60	0 (0%)	1 (1.6%)	1 (1.4%)
	Trend p[b]	0.1428	0.0075	0.0013
Secondary outcomes:				
1. Smoke-free week 6 (US)	10-19	17 (20.2%)	5 (15.6%)	22 (19.0%)
week 24 (Serbia)	20	14 (13.0%)	3 (2.6%)	17 (7.7%)
	21-30	2 (4.4%)	3 (2.7%)	5 (3.2%)
	31-60	1 (7.7%)	1 (1.6%)	2 (2.7%)
	Trend p[b]	0.0160	0.0197	0.0006
2. Smoke-free week 16 (US)	10-19	18 (21.4%)	7 (21.9%)	25 (21.6%)
week 36 (Serbia)	20	12 (11.1%)	6 (5.3%)	18 (8.1%)
	21-30	1 (2.2%)	5 (4.5%)	6 (3.8%)
	31-60	1 (7.7%)	3 (4.9%)	4 (5.4%)
	Trend p[b]	0.0026	0.0274	0.0002
3. Smoke-free week 28 (US)	10-19	13 (15.5%)	12 (37.5%)	25 (21.6%)
week 48 (Serbia)	20	8 (7.4%)	13 (11.4%)	21 (9.5%)
	21-30	2 (4.4%)	11 (9.8%)	13 (8.3%)
	31-60	2 (15.4%)	4 (6.6%)	6 (8.1%)
	Trend p[b]	0.1932	0.0008	0.0003
4. Cessation weeks 25-28 (US)	10-19	10 (11.9%)	9 (28.1%)	19 (16.4%)
weeks 45-48 (Serbia)	20	7 (6.5%)	12 (10.5%)	19 (8.6%)
	21-30	2 (4.4%)	10 (8.9%)	12 (7.6%)
	31-60	1 (7.7%)	3 (4.9%)	4 (5.4%)
	Trend p[b]	0.2176	0.0046	0.0018

5. Cessation weeks 17-28 (US)	10-19	10 (11.9%)	7 (21.9%)	17 (14.7%)
weeks 37-48 (Serbia)	20	6 (5.6%)	7 (6.1%)	13 (5.9%)
	21-30	1 (2.2%)	4 (3.6%)	5 (3.2%)
	31-60	1 (7.7%)	3 (4.9%)	4 (5.4%)
	Trend p	0.1005	0.0138	0.0022

[a] The table shows the number (percentage) of subjects satisfying the criterion for a successful outcome.

[b] Note that trend p is from an exact test; trend p values from chi-squared tests were similar.

Table 7: Testing for possible failure of randomization in the US and Serbian trial[a]

	US		**Serbia**		**Total**	
	Snus	**Placebo**	**Snus**	**Placebo**	**Snus**	**Placebo**
Sex (% male)	35.2	43.2	37.3	39.8	36.4	41.3
Age (mean)	44.3	45.7	43.3	44.0	43.8	44.8
Cigarettes/day in year before baseline (mean)	20.2	20.6	27.6	25.7	24.3	23.5
Age started smoking (mean)	18.1	18.7	19.2	18.8	18.7	18.8
Fagerström dependency test (mean)	5.53	5.58	6.21	6.14	5.91	5.89
Previous quit attempt (% Yes)	89.6	85.6	36.1	36.6	59.7	58.0
Previous use of NRT (% Yes)	55.2	45.6	0.6	1.2	24.7	20.6
Number of subjects	125	125	158	161	283	286

[a] All tests for differences between active and placebo give p values exceeding 0.1

Table 8: Meta-analyses of effects of treatment (intention to treat population)

Outcome		**USA**	**Serbia**	**Total, adjusted for study**
Primary outcome:				
Cessation weeks 6-28 (US)	n[a]	5/2	9/3	14/5
weeks 24-48 (Serbia)	RR	2.50 (0.49-12.65)	3.06 (0.84-11.08)	2.83 (1.03-7.75)
Secondary outcomes 1-5:				
1. Smoke-free week 6 (US)	n[a]	23/11	9/3	32/14
week 24 (Serbia)	RR	2.09 (1.07-4.10)	3.06 (0.84-11.08)	2.27 (1.25-4.12)
2. Smoke-free week 16 (US)	n[a]	22/10	15/6	37/16
week 36 (Serbia)	RR	2.20 (1.09-4.45)	2.55 (1.01-6.40)	2.32 (1.33-4.07)
3. Smoke-free week 28 (US)	n[a]	16/9	25/15	41/24
week 48 (Serbia)	RR	1.78 (0.82-3.87)	1.70 (0.93-3.10)	1.73 (1.07-2.78)
4. Cessation weeks 25-28 (US)	n[a]	13/7	22/12	35/19
weeks 45-48 (Serbia)	RR	1.86 (0.77-4.50)	1.87 (0.96-3.64)	1.86 (1.09-3.18)
5. Cessation weeks 17-28 (US)	n[a]	12/6	15/6	27/12
weeks 37-48 (Serbia)	RR	2.00(0.77-5.16)	2.55 (1.01-6.40)	2.27 (1.17-4.39)
Number of subjects	n[b]	125/125	158/161	283/286

[a] The first number shown is the number of successes in the snus group, and the second is the number in the placebo group.

[b] The first number shown is the number of subjects in the snus group, and the second is the number in the placebo group.

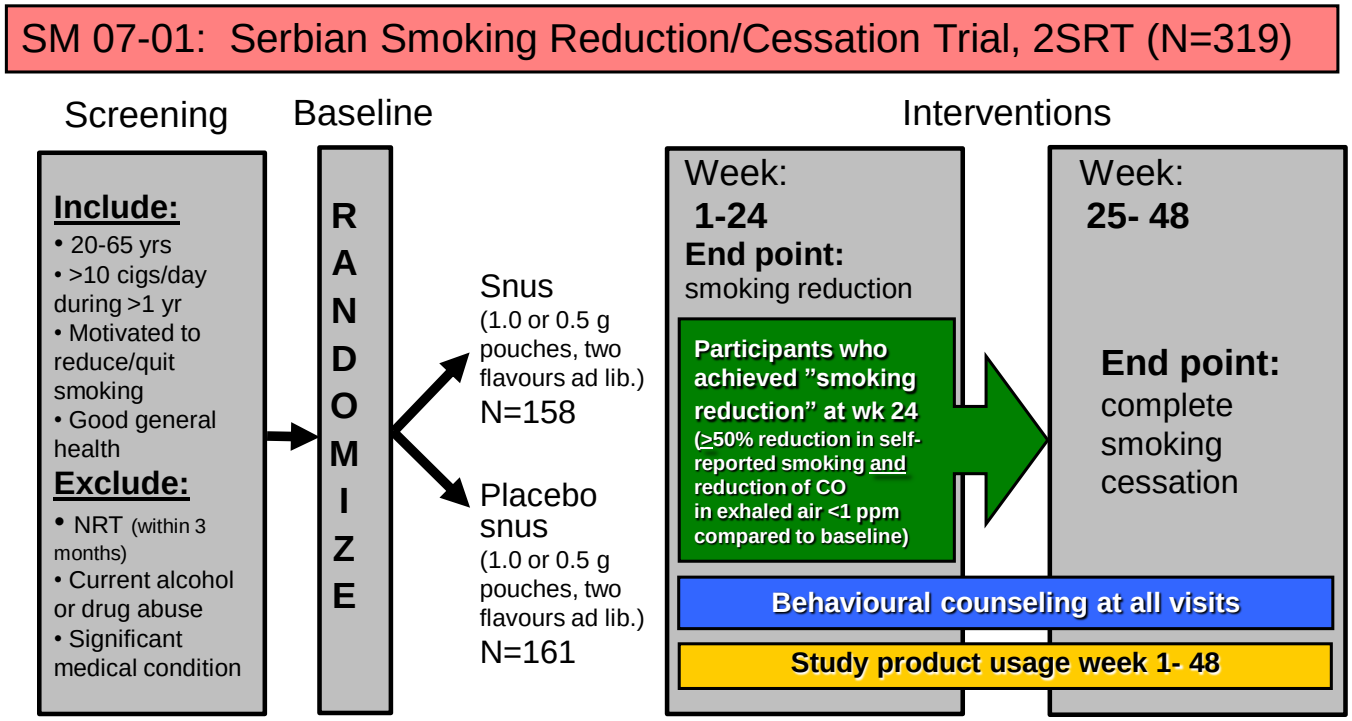
Table 9: Meta-analyses of effects of treatment (compliant subjects)

Outcome		**USA**	**Serbia**	**Total, adjusted for study**
Primary outcome:				
Cessation week 6-28 (USA)	n[a]	5/1	7/3	12/4
weeks 24-48 (Serbia)	RR	5.10 (0.61-42.88)	2.54 (0.67-9.62)	3.09 (1.00-9.55)
Secondary outcome 1-5:				
1. Smoke-free week 6 (US)	n[a]	22/10	6/3	28/13
week 24 (Serbia)	RR	2.24 (1.12-4.49)	2.18 (0.56-8.53)	2.23 (1.20-4.14)
2. Smoke-free week 16 (US)	n[a]	20/9	13/6	35/15
week 36 (Serbia)	RR	2.27 (1.09-4.73)	2.36 (0.93-6.02)	2.30 (1.29-4.11)
3. Smoke-free week 28 (US)	n[a]	16/8	23/14	39/22
week 48 (Serbia)	RR	2.04 (0.91-4.55)	1.79 (0.97-3.32)	1.88 (1.15-3.07)
4. Cessation weeks 25-28 (US)	n[a]	13/6	20/12	33/18
weeks 45-48 (Serbia)	RR	2.21 (0.87-5.58)	1.82 (0.93-3.56)	1.94 (1.13-3.35)
5. Cessation weeks 17-28(US)	n[a]	12/5	13/6	25/11
weeks 37-48 (Serbia)	RR	2.45 (0.90-6.69)	2.36 (0.93-6.02)	2.40 (1.21-4.76)
Number of subjects	n[b]	99/101	122/133	221/234

[a] The first number shown is the number of successes in the active group, and the second is the number in the placebo group

[b] The first number shown is the number of subjects in the active group, and the second is the number in the placebo group

Figure 1: Summary of the Serbian study design



Week	0	1	2	6		12	18	24	30	36	42	48
Visit[1]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CO in exhaled air	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓
Biomarkers[2]	✓					✓		✓		✓		✓
Spirometry	✓					✓		✓		✓		✓
Participant diary (incl. smoking status & study product usage)	--------To be filled in weekly during week 1 through 48----------											

1: Clinical visit or telephone call 2: Blood tests: WBC, CRP, cholesterol, HDL, LDL, fibrinogen, cotinine

Figure 2: Summary of the US study design

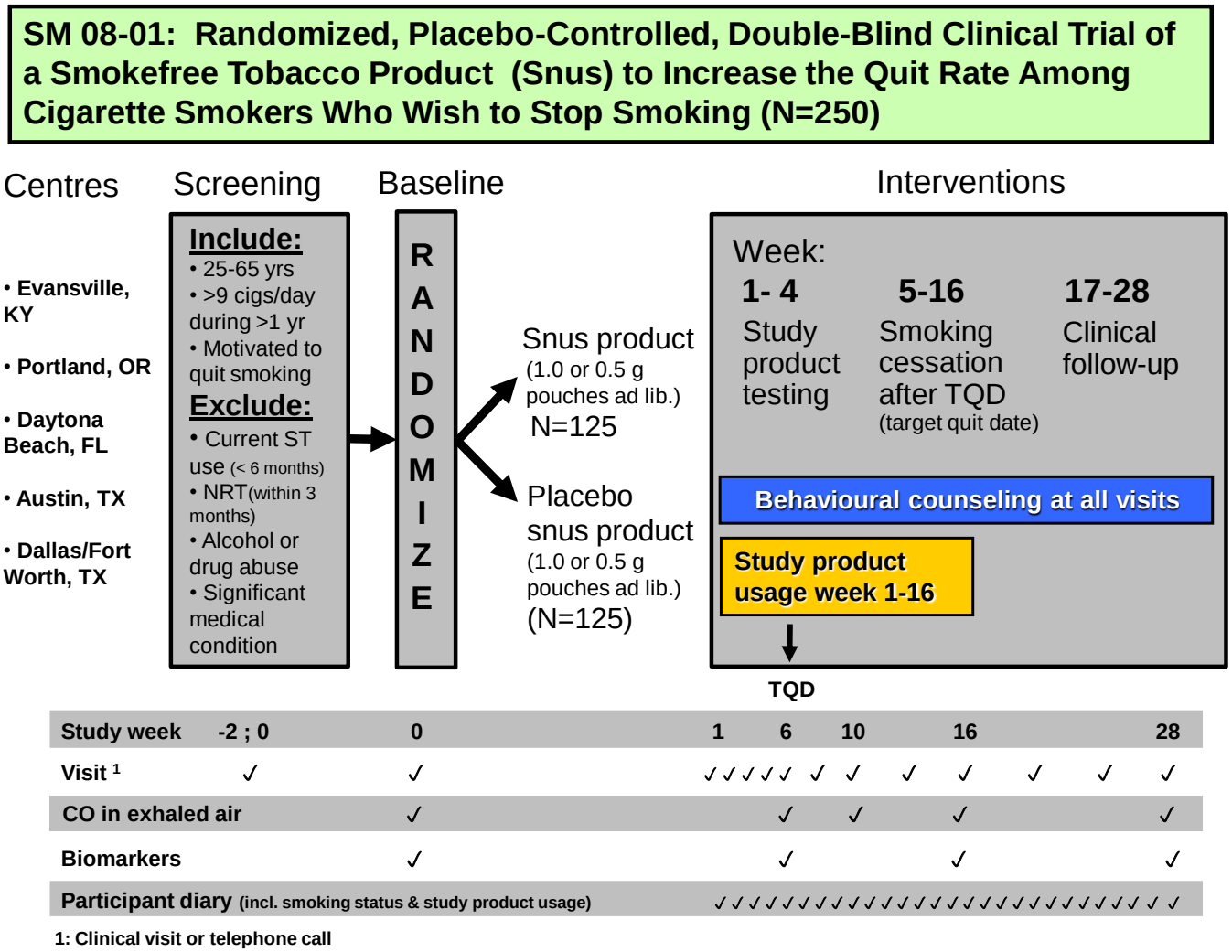


Fig 3: Graphical description of primary and secondary smoking cessation end-points in the meta-analysis of the Serbian and US trials

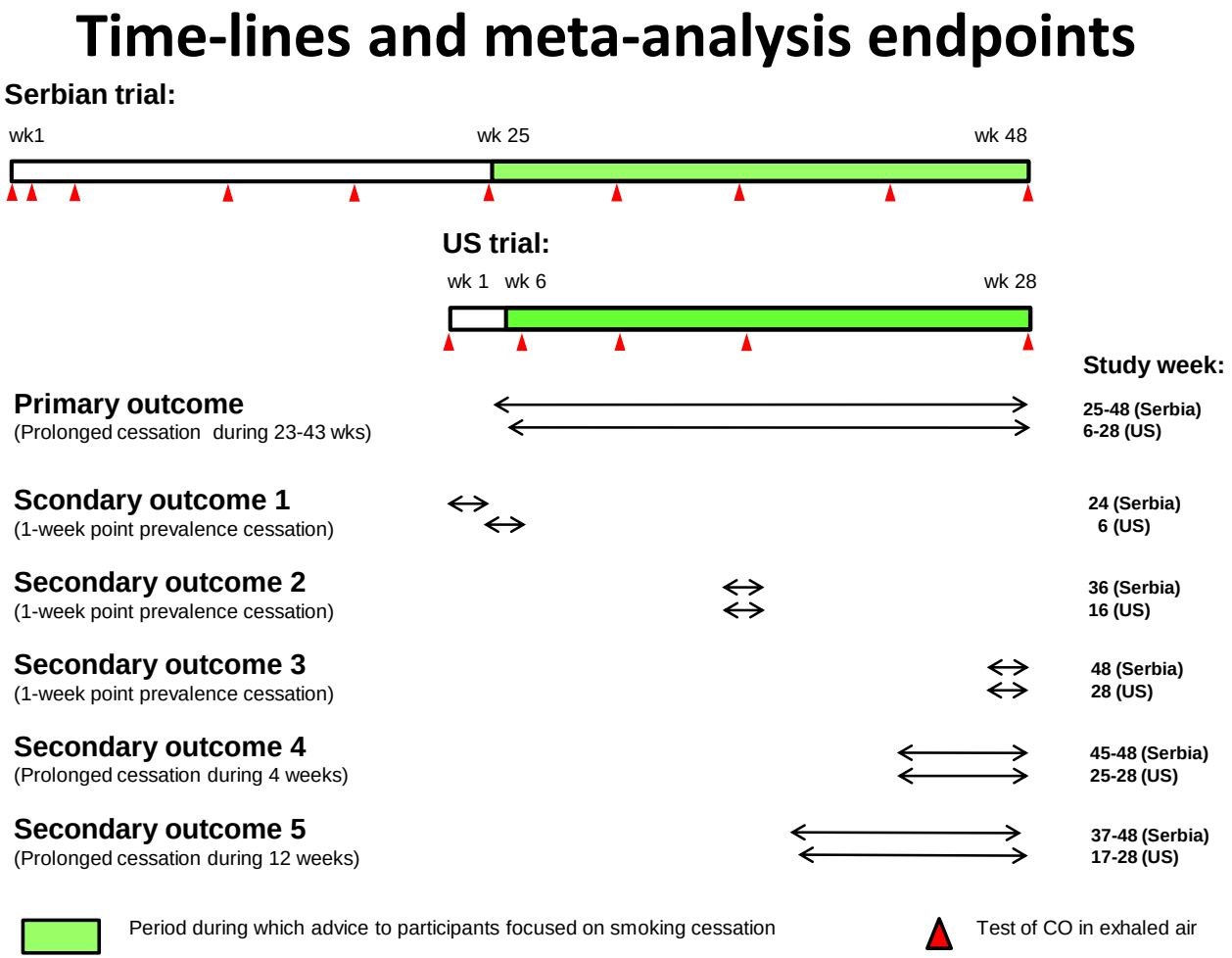


Figure 4:

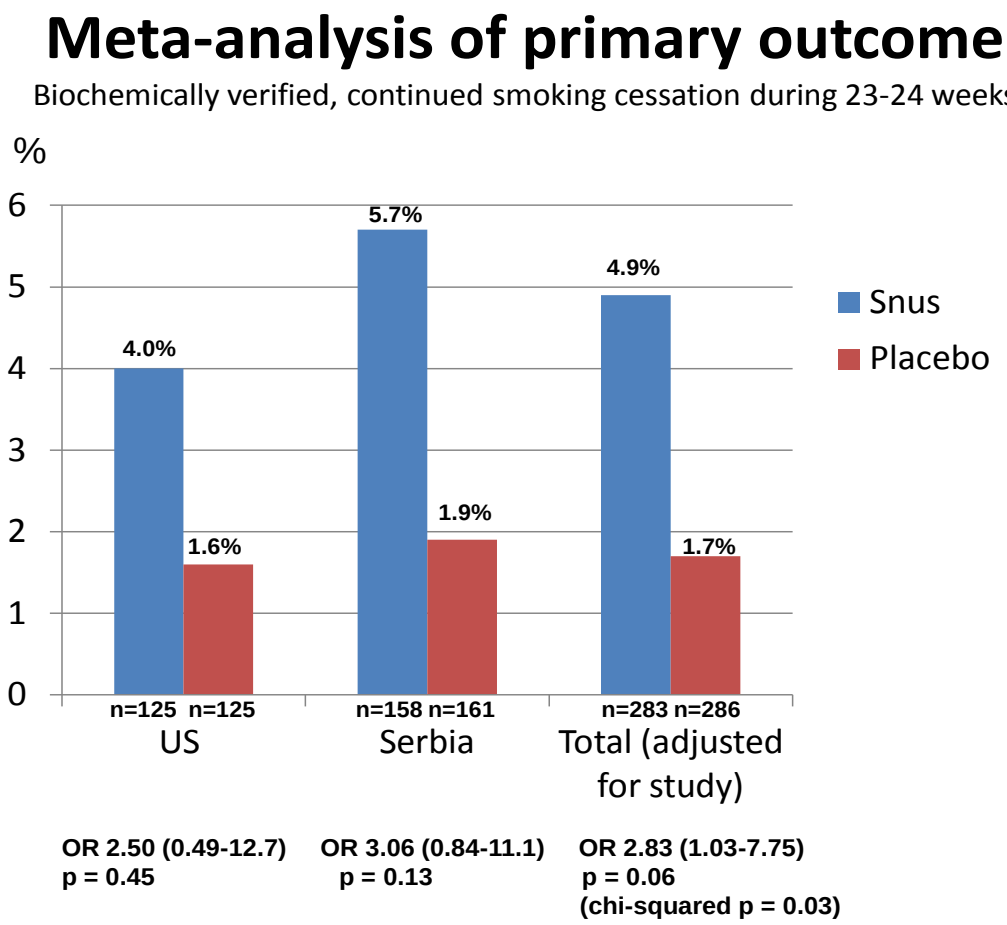

Meta-analysis of primary outcome
Biochemically verified, continued smoking cessation during 23-24 weeks
%
6
5
4
3
2
1
0
4.0%
1.6%
5.7%
1.9%
4.9%
1.7%
Snus
Placebo
n=125 n=125
US
n=158 n=161
Serbia
n=283 n=286
Total (adjusted for study)
OR 2.50 (0.49-12.7)
p = 0.45
OR 3.06 (0.84-11.1)
p = 0.13
OR 2.83 (1.03-7.75)
p = 0.06
(chi-squared p = 0.03)

Figure 5:

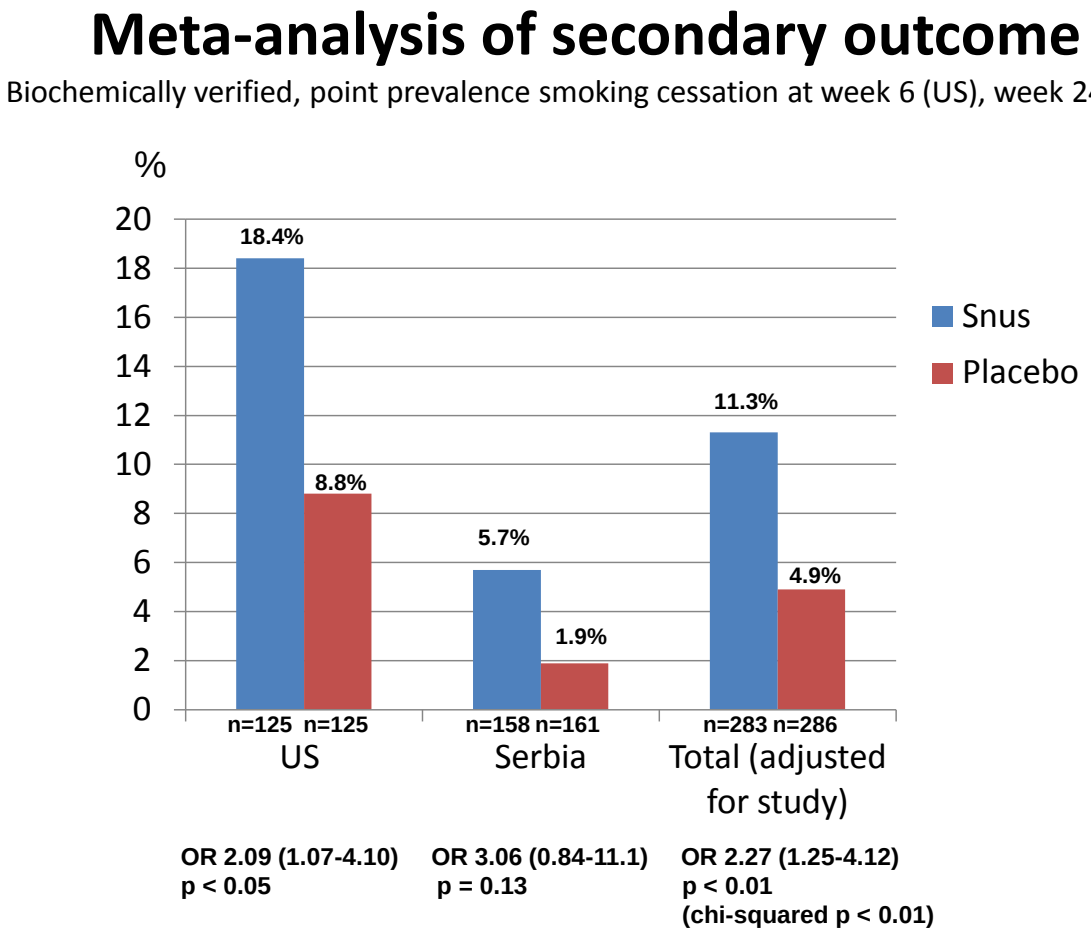

Meta-analysis of secondary outcome 1
Biochemically verified, point prevalence smoking cessation at week 6 (US), week 24 (Serbia)
%
20
18
16
14
12
10
8
6
4
2
0
18.4%
8.8%
5.7%
1.9%
11.3%
4.9%
Snus
Placebo
n=125 n=125
US
n=158 n=161
Serbia
n=283 n=286
Total (adjusted for study)
OR 2.09 (1.07-4.10)
p < 0.05
OR 3.06 (0.84-11.1)
p = 0.13
OR 2.27 (1.25-4.12)
p < 0.01
(chi-squared p < 0.01)

Figure 6:

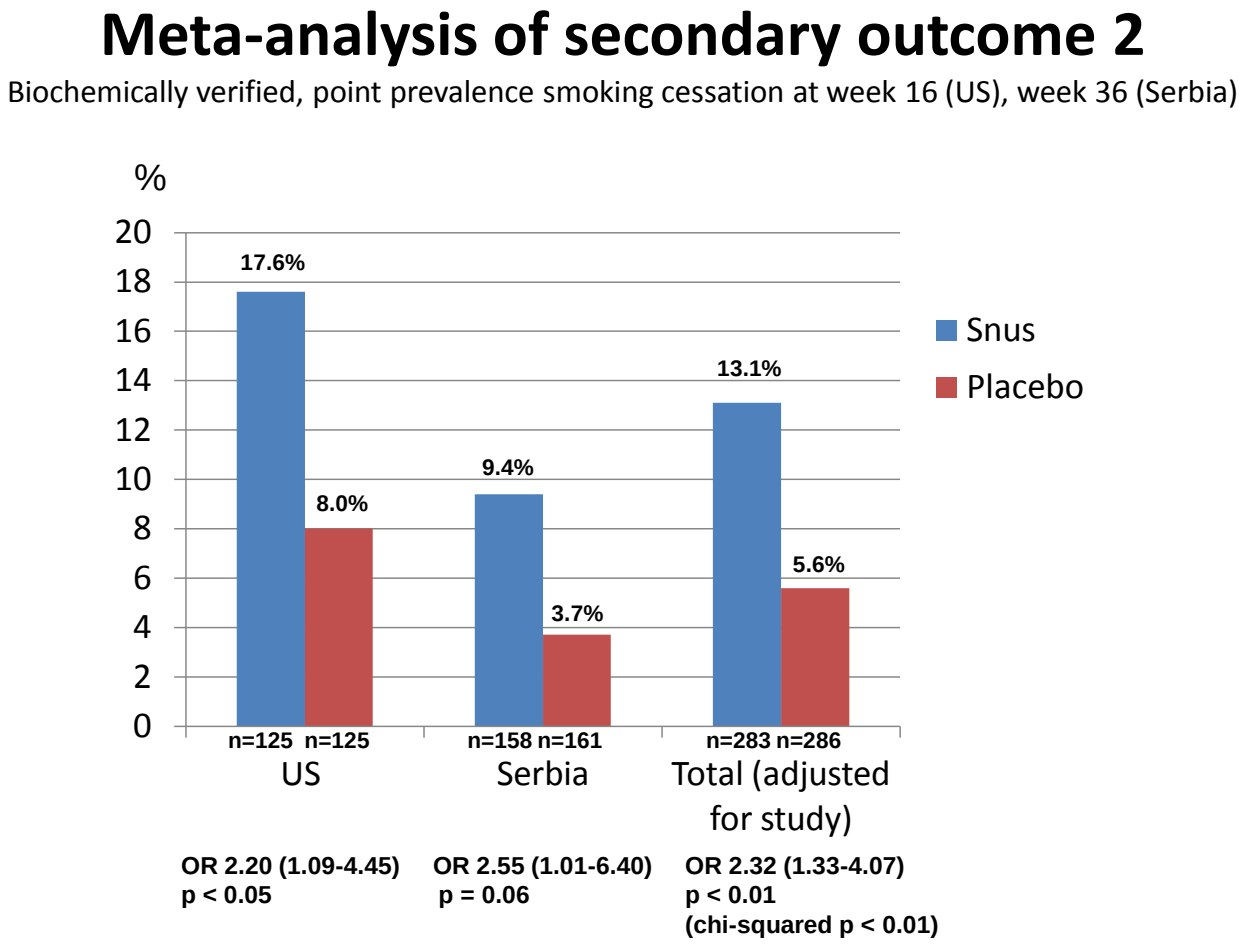

Meta-analysis of secondary outcome 2
Biochemically verified, point prevalence smoking cessation at week 16 (US), week 36 (Serbia)
%
20
18
16
14
12
10
8
6
4
2
0
17.6%
8.0%
9.4%
3.7%
13.1%
5.6%
n=125 n=125
n=158 n=161
n=283 n=286
US
Serbia
Total (adjusted for study)
Snus
Placebo
OR 2.20 (1.09-4.45)
p < 0.05
OR 2.55 (1.01-6.40)
p = 0.06
OR 2.32 (1.33-4.07)
p < 0.01
(chi-squared p < 0.01)

Figure 7:

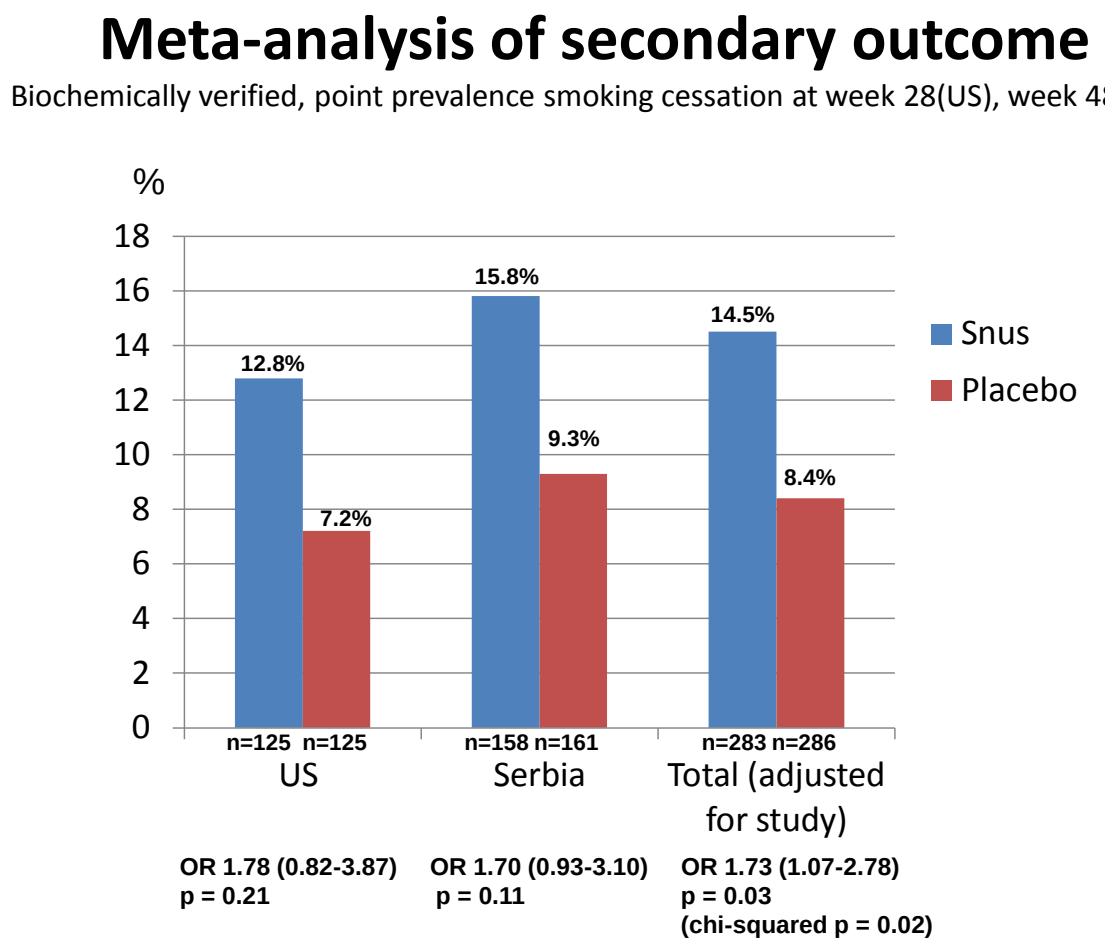

Meta-analysis of secondary outcome 3
Biochemically verified, point prevalence smoking cessation at week 28(US), week 48 (Serbia)
%
18
16
14
12
10
8
6
4
2
0
12.8%
7.2%
15.8%
9.3%
14.5%
8.4%
Snus
Placebo
n=125 n=125
US
n=158 n=161
Serbia
n=283 n=286
Total (adjusted for study)
OR 1.78 (0.82-3.87)
p = 0.21
OR 1.70 (0.93-3.10)
p = 0.11
OR 1.73 (1.07-2.78)
p = 0.03
(chi-squared p = 0.02)

Figure 8:

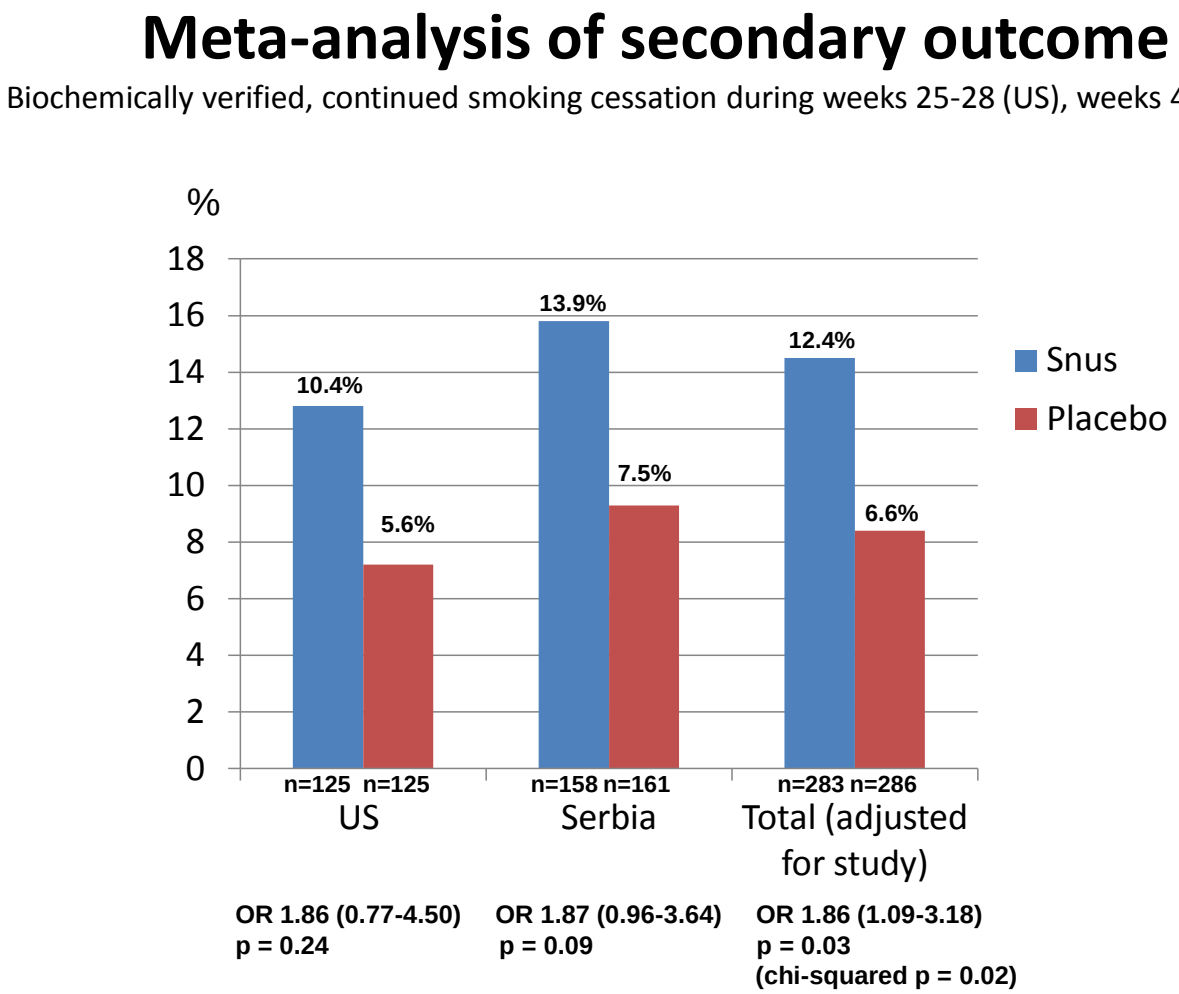

Meta-analysis of secondary outcome 4
Biochemically verified, continued smoking cessation during weeks 25-28 (US), weeks 45-48 (Serbia)
%
18
16
14
12
10
8
6
4
2
0
10.4%
5.6%
13.9%
7.5%
12.4%
6.6%
Snus
Placebo
n=125 n=125
US
n=158 n=161
Serbia
n=283 n=286
Total (adjusted for study)
OR 1.86 (0.77-4.50)
p = 0.24
OR 1.87 (0.96-3.64)
p = 0.09
OR 1.86 (1.09-3.18)
p = 0.03
(chi-squared p = 0.02)

Figure 9:

Meta-analysis of secondary outcome 5

Biochemically verified, continued smoking cessation during weeks 17-28 (US), weeks 37-48 (Serbia)