MODELS FOR TOBACCO PRODUCT EVALUATION

PROJECT 4: CLINICAL TRIAL METHODS FOR ASSESSING A TOBACCO PRODUCT

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Changes to design after funding (duration to 8 weeks and clarifications to protocol).	1/15/2013	2/28/2013	1/7/2013
Additional biomarker collections, CoC, amend eligibility criteria, other clarifications/changes	8/22/2013	9/12/2013	8/25/2013
Correct compensation amounts; add usual brand urine; change advertisement; eligibility includes 5-10 cig/day; NicAlert of 6	10/15/2013	1/9/2014	10/15/2013
Drop Sampling Phase criteria from 14 to 7 snus	10/17/2013	11/18/2013	NA

1.0 INTRODUCTION AND OVERVIEW

A historic event for tobacco control in the United States was the passage of the Family Smoking Prevention and Tobacco Control Act (FSPTCA) that gave the Food and Drug Administration (FDA) jurisdiction over the regulation of tobacco products. The FSPTCA allows the FDA to establish tobacco product performance standards (e.g., limits on yields of constituents in tobacco products including nicotine). Also, the FDA will evaluate the evidence to support health claims for modified risk tobacco products (MRTPs), that is, "any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products". Similar regulatory efforts are occurring worldwide. For any decisions or actions in these areas, the FDA and others will need scientific data to promulgate regulations that result in improved public health. The conduct of regulatory science presents a challenge because there are many gaps in our knowledge about the best methods for conducting human studies to determine how the products will be used and the resulting toxicant exposure and to evaluate the validity of health claims (3-7). The fundamental methodological issues that need to be addressed include methods for recruiting a sample representative tobacco users who are interested in using the tobacco product; the effects of the subject's perception of the product on uptake and use; the effects of instructions for product use on the pattern of use and toxicant exposure; the change in responses to the tobacco product over time; and the identification of valid biomarkers of exposure and effect. Typically, these areas are investigated independently, yet it is the integration of findings that is necessary to accurately assess the impact of a tobacco product. In this project, we will enroll 690 subjects into a 8-week, 5-arm trial associated with varying instructions for use of an oral non-combustible tobacco product, Camel Snus, as a means to assess product substitution and dual use.

2.0 SPECIFIC AIMS

Primary Aims:

1. To compare product use patterns and biomarkers of exposure and effect based on differing instructions for oral tobacco use: a) complete substitution vs. partial substitution for cigarettes; and b) specific instructions for use vs. no instructions (*ad libitum* use).
2. To determine stabilization of product use by examining time effects for subjective (e.g., dependence), biological (e.g., biomarkers of exposure) and behavioral responses (e.g., smoking exposure and oral tobacco topography).
3. To determine important moderators of use of a product and exposure (i.e., consumer perception of the product and consumer response to the product after sampling [Project 3], nicotine metabolite ratio, nicotine dependence, gender, race and age).

Secondary Aim:

4. To develop and test cost-efficient and rapid measures to assess levels of exposure from oral tobacco products (determining exposure based on used oral tobacco products) and cigarettes (examining solanesol levels from cigarette filters).

Hypotheses

Primary Aims:

1. Instructions for use will lead to significant differences in patterns of use, which will be related to biomarkers of exposure.
2. Time in study will have an effect on outcome measures and will reach a plateau by at least 6 weeks.
3. Moderators for exposure will include pattern and intensity of tobacco product use. Pattern and intensity of product use will be affected by subjective responses to products,

perception of safety of the product, gender, race, age, degree of dependence and nicotine metabolism phenotype.

Secondary Aim:

4. Biomarkers of exposure will be related to levels of chemical constituents extracted from used oral tobacco pouch products in the complete substitution condition, plus levels of solanesol in filters in the partial substitution condition.

The outcome of this study will provide guidance on methods and measures that should be used in clinical trial assessments of tobacco products (e.g, MRTPs or new tobacco products). Furthermore, this study will novelly consider the interactions and effects of instructions of product use, perception and response to the product, and individual differences on product uptake and use and toxicant exposure.

3.0 SIGNIFICANCE AND BACKGROUND

We are experiencing an unprecedented time when we have a global convention for tobacco control (8) and in the U.S., federal governmental regulation over tobacco products (9). Two important components of both these tobacco control measures include: 1) governmental authority to mandate tobacco product performance standards, such as levels of chemical constituent yields and smoke emissions and toxicity; and 2) governmental requirement to evaluate exposure and health risk claims for tobacco products, including the so called modified risk tobacco products (MRTPs) to assure that the claims are evidence-based and consumers are not misled (e.g., in the Articles 9-11 of the FCTC and Sections 907 and 910 of the FSPCA). Pivotal to the work of regulators is sound scientific evidence that is sufficient to make policy decisions with the goal of improving public health and/or minimizing public harm. The FDA must also be able to defend their actions to the public, legislators and the courts. This scientific evidence relies on the use of valid methods and measures to assess tobacco products. The importance of having these assessment tools is clearly evident from the experiences that we have had with lower yield cigarettes in the 1970s and 1980s. Because of the limited scientific knowledge and tools that we had possessed at the time, erroneous assumptions were made about “lights” and their assumed reduced toxicant exposure compared to regular yield cigarettes. Only decades later did we learn that smokers do not smoke like machines, biomarkers that measure toxicant exposure are similar regardless of the yield of cigarettes, and cancer rates did not decrease with the decrease in tar yields of cigarettes (10). With the influx of new MRTPs into the market intended by tobacco manufacturers to retain tobacco consumers, and the future possibility of tobacco product standards, the need for rigorous and valid methods to assess tobacco products has become imperative.

An essential component of tobacco product assessment is human clinical trials (3, 11). Only human studies provide sufficient evidence about human tobacco use and exposure. The goals of these trials are to determine how the products are used, the extent of exposure to toxicants and the risk for disease with the use of these products. As part of a National Cancer Institute contract (N01-PC-64402), we conducted an extensive review of the literature on clinical trial methods and measures for tobacco products, specifically MRTPs. We also held a workshop on this topic with experts in the field of tobacco product evaluation, clinical trial methods, abuse liability and consumer perception testing, and subsequently published an article that provided recommendations for the conduct of these trials and that identified research gaps (4). To date, human forced-switching studies have been one of the primary methods used to examine the toxicant exposure associated with the use of specific tobacco products including MRTPs. These studies typically involve randomization to one or more MRTPs and use of conventional cigarettes and/or no smoking with or without nicotine replacements as the control group(s) in a parallel arm design. Despite these studies, we concluded that the best trial methods for

evaluating MRTPs are still uncertain. For example, prior MRTP studies have varied in their instructions to subjects for product use (4, 7). Studies have required complete or partial substitution of cigarettes with the assigned product and they have provided either instructions for specific amounts of product use or *ad libitum* use of the products (no required amounts of product use). These varied instructional methods can answer different questions regarding the toxicant exposure associated with use of a MRTP. Instructions for complete substitution may be more reflective of the toxicity associated with the product itself, whereas partial substitution may be more reflective of how consumers will tend to use the products in the "real world" setting. Most MRTP studies have employed complete substitution and only a few studies have used partial switching of MRTPs (4, 13), although there are several partial substitution studies that have been conducted with nicotine replacement products (14). Partial substitution studies using nicotine replacements and using potential modified risk tobacco products show a reduction in cigarette consumption compared to baseline levels or continued smoking groups (13-16). Separately, studies employing *ad libitum* vs. specified amounts of use have been found to differentially affect biomarkers of exposure in a naturalistic setting (e.g., 17, 18), but not in a controlled residential setting (19). These results suggest that the study setting, the instructions for how to use the product and amount of product use may influence outcomes measures. To date, no study has directly compared the effects of these varied instructions on toxicant exposure.

Another critical methodological issue that has not been addressed is the time to stabilization of product use and whether assessment of a product during the initial phase of use differs from assessments made after several weeks of use. Examining the effects of duration of use on biological, behavioral and subjective outcome measures is important because MRTP study lengths have ranged from one day to one year (4), and if stabilization of use patterns have not occurred, then only incomplete information is being learned. Recent studies conducted by tobacco industry scientists Roethig et al. (20) and Mendes et al. (21) suggest that stabilization may occur in the first few weeks of product switching, although no systematic study across different measures has been conducted. If stabilization occurs within a few weeks, then clinical trials assessing product use and exposure only need to be this short duration, reducing cost and subject burden, and enhancing compliance.

A third critical methodological question that has been inadequately addressed is the identification of individual factors that may moderate exposure. Examining these factors is important in order to identify variables that significantly influence the uptake of a product, how the product is used and extent of toxicant exposure. These data will help us to understand how a tobacco product might differentially affect subpopulations of smokers. It will also help determine the essential recruitment criteria for subjects in future studies that are examining these outcome variables (4). For example, if age is an important moderator variable, then future studies should have sampling strategies that account for age.

In the proposed study, oral non-combusted tobacco, Camel Snus, has been chosen as the product to examine the above methodological issues associated with forced-switching studies. The proposed use of oral non-combusted tobacco products as a means of reducing harm in cigarette smokers has engendered a great deal of controversy. Most public health officials agree that exclusive use of smokeless tobacco would be associated with less disease risk than smoking cigarettes and on an individual risk basis, is most likely to result in harm reduction compared to other modified combustible tobacco-containing products (22). The risk of disease associated with smokeless tobacco use has been estimated to be 2% the mortality risk of cigarette smoking (23, 24). If smokers completely switched to smokeless tobacco use, then the relative risk of disease would likely be dramatically reduced. In Sweden, the increased uptake of smokeless tobacco or snus has been hypothesized to explain the decrease in cigarette smoking among men with a consequent decrease in lung cancer mortality (25). Studies also

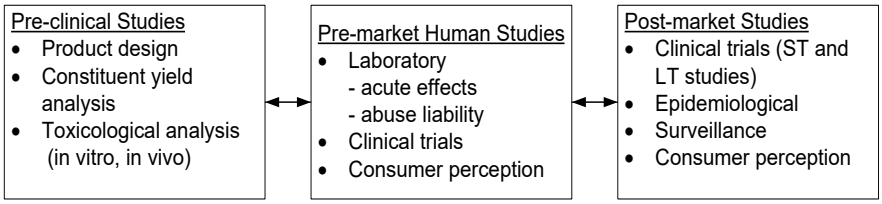
have shown that when smokers switch to oral tobacco products, a significant decrease in several biomarkers of exposure and effect are observed (2, 26), largely due to reduced delivery of toxicants that otherwise are produced by combustion when smoking.

The urgency to examine oral tobacco products is driven by a number of important factors: 1) all the major cigarette companies have become important players in the smokeless tobacco market and are promoting these products as an alternative to smoking, or for use in situations where smoking is not permitted (e.g., workplaces, bars or restaurants); 2) some tobacco control advocates believe that the Swedish experience can be replicated in other countries and will lead to reduction in tobacco-related harm; 3) the extent of human toxicant and nicotine exposure from many smokeless tobacco products is unknown; and 4) patterns of use of these products are unknown, and smokers may engage in dual product use such that level of exposure to toxicants may in fact not reduce but increase (e.g., by maintaining or increasing nicotine dependence). Because of these factors, oral tobacco products will serve as the vehicle to investigate the methodological issues.

Because we are proposing to use an oral tobacco product, we will also be examining and testing measures of exposure. For example, for oral tobacco products, determining the effects of oral tobacco topography and its relation to exposure biomarkers has been limited to a few studies (27, 28). Novel methods, such as determining the amount of tobacco constituents extracted after use and relating these findings to biomarkers of exposure of these constituents, have never been explored, yet may prove to be cost-efficient compared to biomarker assessments. Similarly, measuring the solanesol levels, a tobacco-specific compound in cigarette filters, as an indicator of exposure has shown promise and may be another cost-efficient, rapid and simple measure of exposure, but requires further validation studies (29-31).

Conceptual framework for tobacco product evaluation. Clinical trials are a critical component of tobacco product evaluation (5, 32). Figure 1 shows a framework for the evaluation of tobacco products.

Figure 1. Conceptual Framework for Tobacco Product Evaluation



The primary goals of pre-clinical non-human and pre-market human product evaluation studies are to determine the toxicity of a product, its abuse liability (e.g., uptake and dependence producing potential), and consumer perception of the product that influences uptake and continued use (e.g., consumer reaction to promotion, packaging and price). Another major goal is to determine the use of the product and consequent extent of exposure to toxicants and ensuing health risks. The findings from these assessments will then be evaluated for potential individual and population risks and benefits should the product be released into the market or should a manufacturer make specific claims for reduced toxicant exposure of reduced health risks. Once introduced into the market with or without specific claims, then post-market analysis will be conducted to monitor and assess for public health harm (or even benefit). While this Project will be conducting studies to examine methods for clinical trials, other Projects and Core B in this U19 will be conducting constituent analysis to assess levels of toxicants, studies examining animal and human models for assessing abuse liability and studies examining methods for assessing consumer perception and response. The findings from these Projects will be integrated to determine the interrelationships of the findings and to identify strengths, limitations and gaps of the conceptual framework. As described in Core A, the potential risks and benefits of the product on the

population as a whole will be assessed using modeling techniques that have been employed by Dr. David Levy, who is a member of our Scientific and Policy Advisory Committee. Dr. David Levy has developed SimSmoke, which is a system based simulation model that can used to predict the potential impact of tobacco policies on smoking behavior and health outcomes (33) This model would be ideal to examine the population effects of introducing a modified risk tobacco product into the market. The advantage of this model is that it takes into account other tobacco policies that would surround the introduction of a product onto the market, such as smoke-free air laws, the price of cigarettes, the price of snus product, marketing and labeling of tobacco products, mass media campaigns, youth access laws, and the availability, uptake and effectiveness of tobacco cessation services. The model also takes into account long-term trends in smoking or smokeless tobacco use as well as population changes through birth and death. Undertaking this exercise will also help us to identify weaknesses and strengths in our conceptual framework for tobacco testing by identifying information gaps.

In summary, this research project will provide researchers with fundamental data to design and conduct switching studies for tobacco products. It will provide governmental agencies guidelines for evaluating research studies and their significance. This project will also provide insight into the extent of toxicant exposure and potential health effect from lower nitrosamine, oral tobacco products using novel modeling methods.

Overview of Experimental Design:

This is a multi-center trial involving University of Minnesota, Ohio State University and Roswell Park Cancer Center. Subjects who are eligible will enter a Camel Snus sampling phase (N=793). Smokers (estimated N=690) interested in continuing with the study after the sampling phase will undergo a 2 week baseline assessment phase and will then be randomized in the clinical trial phase to one of five experimental conditions for 8 weeks. Tobacco use patterns, subjective responses to product, and nicotine and toxicant exposure will be assessed.

6.0 STUDY PROCEDURES

6.2 Subject Selection:

Cigarette smokers (n=793) will be recruited at multisite locations.

Recruitment plan:
Subjects will be recruited from advertisements through a variety of media outlets and the internet. A variety of media will be used that will foster the recruitment across a spectrum of age, education and socioeconomic status, race/ethnicity, and light and heavy smoking. Cigarette smokers will contact the respective clinical trial centers and be screened for eligibility over the telephone.

The advertisement would read as follows:

Smokers who want to try a new oral tobacco product developed for smokers are needed for a research study that may reduce their exposure to harmful tobacco smoke.

(The content of this advertisement was discussed amongst investigators and the NCI Contract Expert Consulting Committee and was considered informative and not misleading.)

Eligibility Criteria
Subjects must meet the following criteria for eligibility:
The inclusion criteria are the following:

a) Male or female subjects who are at least 18 years of age;
b) Smoking history of $\geq$10 cigarettes daily and CO$\geq$10 ppm or if CO <10 ppm a NicAlert equal to6 (over ~1000 ng/mL cotinine concentration in urine). Subjects who smoke >5 to 9 cigarettes per day will be eligible, but their NicAlert level must be equal to 6;
c) Smoking daily for at least 1 year and no serious quit attempts in the last 3 months (to ensure stability of daily smoking, particularly for those randomized to the continued smoking group);
d) No unstable and significant medical or psychiatric conditions as determined by medical history and Prime-MD (to ensure safety of the subject, to minimize the effects of poor health on biomarker measures and to maximize compliance to study procedures);
e) Lack of stabilization of medications (determined by study MD);
f) Subject has provided written informed consent to participate in the study (adolescents under the age of 18 will be excluded because this project involves continued use of tobacco products and new tobacco products);

The exclusion criteria are the following:

a) Immune system disorders, respiratory diseases, kidney or liver diseases or any other medical disorders that may affect biomarker data;
b) Current or recent (<3 months) alcohol or drug abuse problems;
c) Regular tobacco use (e.g., greater than weekly or 9 days in last 30 days) other than cigarettes;

d) Use of smokeless tobacco products within the last 3 months;
e) Use of roll your own cigarettes that are unfiltered or not machine rolled;
f) Currently using nicotine replacement or other tobacco cessation products (to minimize confounding effects of another product) or intention to quit in next three months;
g) Pregnant or breastfeeding (due to toxic effects from tobacco products).
h) Unable to read for comprehension or completion of study documents.
i) *Clinical Trial Additional Exclusion Criteria:* Regular substance use (other than alcohol) defined as use of a substance 15 of the last 30 days. This will be assessed via the Drug Use Questionniare-1month.

If subjects meet the eligibility criteria for the study, they will be asked to attend an orientation meeting where the entire study will be explained in detail, informed consent will be obtained and the Screening Questionnaires will be completed (Appendix A, Scales and Subjective Measures). Pregnancy exclusion will be confirmed through a urine test.

6.3 Randomization:
Randomization will be stratified by site and number of cigarettes smoked per day (≤ 18/day vs. > 18/day, the expected median number of cigarettes smoked per day) to assure balance across treatment groups. Treatment groups will be checked for balance on other factors post randomization and any imbalances will be accounted for in our analysis as described in the statistical analysis section.

6.4 Study Procedures

Sampling Phase:
The purpose of this phase will be to gauge product acceptance, reduce dropouts in the clinical trial and recruit subjects in the clinical trial that may be more representative of those who might purchase and use the product. Subjects will be provided the product for one week to determine palatability, sensory and consumer perception and initial pattern of product use. Subjects will return to the clinic after one week to determine their interest in continuing in the study.

During the orientation visit of this sampling phase, we will collect data that may influence the subject's perception of the product. These measures include basic demographic data and smoking history, consistent with questions used in the Tobacco Use Supplement to the 2010 Current Population Survey, and in the Population Assessment of Tobacco and Health (PATH) Survey allowing comparisons with a nationally representative sample of smokers. Additionally, we will use the Fagerstrom Test for Nicotine Dependence (FTND), Wisconsin Inventory of Smoking Dependence Motives (WISDM), Cigarette Evaluation Scale, Stages of Change scale PrimeMD, CES-D, Short Michigan Alcohol Screening Test (SMAST) and Drug Abuse Screening Test (DAST) and Perceived Health Risk of cigarettes. At the orientation visit, subjects will be instructed on how to use the product and presented with the Winterchill and Robust flavor snus tins. They will be instructed to smell the product and choose a flavor to try during the visit. After smelling the product, subjects will rate the sensory perception, taste and appeal of the product immediately after this initial exposure using the Odor/Haptic Scale (see Project 3). Afterward, the subject will place the snus pouch between their lip and gum for a 5 minute period and then expectorate the pouch. Subjects will complete the Product Evaluation Scale (PES), Drug Effects/Liking Questionnaire, Tobacco Purchase Task and Perceived Health Risk after expectorating the snus. Participants will then be provided Camel Snus to use at home (4 tins @ 15 pouches per tin) and told to "Use the product as you wish over the next week. If subjects want more, they can return to the clinic for a maximum of 14 tins/week. Camel Snus was

chosen because it is now nationally marketed, and based on our pilot study, has levels of nicotine similar to a 4 mg nicotine lozenge and appears to be palatable to smokers. Participants will complete a daily diary via IVR about the amount of snus, cigarette or any other nicotine-containing product use. .

After 1 week when subjects return to the clinic, we will assess: 1) number of cigarettes, snus sachets, and other nicotine products used during the past 7 days; 2) responses to the Product Evaluation Scale, Drug Effects/Liking Questionnaire and the Perception of Health Risks; 3) consumer perception measures that examine risks, benefits and feelings associated with the product; 4) willingness/unwillingness to participate in the experimental phase of the study; 6) interest in switching from cigarettes to the Camel Snus if it were not for the study; 7) impact on interest in quitting tobacco use; and 8) interest in paying for the product and the amount the subject is willing to pay for the product to replace cigarette smoking (measures from Project 2). Compensation for Sample Phase: Subjects will receive $5 for transportation, $5 for IVR completion, and $40 for trying the study product for 1 week for a total $50, whether they want to continue with the study or not.

Clinical Trial Phase:

At the end of the sampling phase, eligibility for the clinical trial phase will be determined. Eligible subjects will have used an average of at least 2 sachets of Camel Snus daily during the sampling phase or at least 7 snus sachets used over most days during the sampling period. Subjects must be willing to attend clinic visits weekly for 4 weeks and biweekly visits for 6 weeks and a follow-up visit at 12 weeks post study product use and agree to be called at 4 and 24 weeks post-study. Subjects who wish to continue in the study will undergo 2 weeks of baseline where they will resume smoking and then will be randomized to one of five experimental conditions.

Baseline smoking period*:*

Subjects will be required to attend baseline clinic visits during Week -2 (visit 91) and -1 (visit 00) of the clinical trial phase, and they will record their cigarette or other tobacco intake on a daily basis using an interactive voice response system (IVR) (see Table 1).

First baseline visit: This baseline visit will assess tobacco use status to determine if smoking has returned to levels prior to sampling phase. Subjects will collect a first morning void urine sample and bring it in on the day of their clinic visit for study biomarkers.

24 hours prior to second baseline visit: Prior to the second baseline visit, subjects will collect all of their cigarette butts in a container provided for the entire day.

Second baseline visit*:* Subjects will collect a first morning void urine sample on the day of their clinic visit. The 24 hour cigarette butt collection and urine sample will be brought with them to their clinic visit. Blood samples will be drawn for study biomarkers Buccal cells will be collected 20 minutes after the subject brushes their teeth using a cytobrush rubbed against the buccal mucosa as per previous studies (34). Oxygen saturation, CO, vitals and weight will be obtained. Subjective measures will include predictors for MRTPs use, factors that may influence the biomarker measures, and outcome measures (see Tables 1 and 2).

Experimental period:

After the baseline assessment, subjects will be randomized to one of five experimental conditions: 1) smoking usual brand cigarette controls, who after 8-weeks will be offered Camel Snus and instructed for partial or complete substitution of cigarettes (subject's choice); 2) complete substitution (i.e., no smoking) and *ad libitum* use of snus; 3) complete substitution (i.e., no smoking) and specific instructions for snus use based on the number of cigarettes smoked per day (if 10-20 cigarettes, use 8 pouches per day snus, and if >20 cigarettes then 12

pouches per day – these amounts were derived from a clinical trials conducted by us and by Eissenberg and associates [personal communication]). 4) partial substitution with *ad libitum* use of both snus and cigarettes; and 5) partial substitution with controlled use of snus (paralleling the instructions for complete substitution condition) and *ad libitum* smoking. All subjects will be instructed to use the snus pouch for at least 5 minutes and optimally 30 minutes per occasion; Snus, but not cigarettes, will be provided to subjects at the clinic visits.

Procedures for clinic visits: Subjects will continue to complete daily diaries for tobacco and other nicotine product use. At every clinic visit, vital signs, weight, oxygen saturation and expired air CO will be obtained. At weeks 2, 4, and 8 of product use, blood, and first morning void urine collection samples will be collected. At week 6, a first morning void urine sample will be provided. Buccal cells will be collected at weeks 4 and 8. Samples from weeks 4 and 8 will be analyzed for biomarkers as described below. Samples taken at week 2 will be aliquoted and stored for future analyses governed by results from the 4 and 8 week analyses or for other future studies. Questionnaires that measure factors that may influence biomarker assessments will be administered at the time of biosample collection. All other subjective measures will be collected thoughout the experimental phase. All cigarette butts, as appropriate for the study group, will be collected in a container 24 hours before the week 4 biomarker clinic visits. In addition, used and unused snus will be collected for 24 hours prior to week 4 to assess for amount of constituents that have been extracted. During this collection, time in and time out of snus products via a paper diary will be recorded to assess oral tobacco topography. At the end of experimental phase, all subjects will be strongly encouraged to quit using all tobaco products and set a quit date. A treatment manual (Clearing the Air) and treatment resources will be provided.

Smoking Usual Brand Condition. Subjects in this condition will undergo all the procedures as those subjects assigned to Camel Snus, except they will be told to smoke their usual brand of cigarettes and follow their normal patterns. At the end of the 8-week period, they will be offered the product for up to 8 weeks, with a choice of how they want to use the product. They will return to the clinic at 9, 10 and 12 weeks for assessment of use patterns (1, 2 and 4 weeks on the product). During this period, subjects will keep daily diaries via Interactive Voice Response (IVR). After 4 weeks of product use, they will come to the clinic whenever they want products for up to 8 weeks. At these clinic visits, their tobacco use patterns will be assessed via the Daily Use Summary and subjective forms. At the end of the 8 weeks (Week 16) of snus use, subjects will bring in a first morning void urine sample for analysis of tobacco exposure measures. Subjects will be paid $50 for the Week 16 visit. Follow-up will occur as described below after the 8-week product access period (Weeks 20, 28 and 40). If subjects in the Usual Brand Condition do not choose to use the Camel Snus, they will follow the Follow-up schedule for the snus arms.

Follow-up. Follow-up clinic visit will occur for all subjects at week 20 (12 weeks after the end of the trial period). Tobacco use status (amount and type of tobacco product use) will be determined. CO will be obtained and a first morning urine and blood sample. These samples will be stored for cotinine assessment. Follow-up calls will occur at week 12 and 32 (4 and 24-weeks post-study) and tobacco use will be assessed. Those continuing to smoke or use Camel Snus will be strongly advised to quit and treatment resources will be provided.

Product Compliance Sessions. At each visit, subjects will be asked and counseled about their use of Camel Snus. The procedures for inquiry and counseling will be standardized. An intervention manual for each of the conditions will be developed.

- *Complete Substitution Condition*: Subjects will be asked about any concerns or obstacles associated with only using Camel Snus. If difficulties are encountered, then subjects will be asked about why they think they are experiencing difficulties (e.g., taste,

lack of satisfaction, withdrawal symptoms) and will be asked to problem solve how to deal with these difficulties so that they can meet the requirements of the protocol.

- *Partial Substitution Condition:* Subjects will be asked about their experience with using Camel Snus. Subjects in the *ad libitum* condition will be encouraged to use as much or as litte as they want. Subjects in the other condition will be encouraged to follow specific instructions for use.

Product compliance. The importance of honest reporting will be stressed to the subjects. Compliance to the instructions for product use will be determined by: a) daily diary records; b) tobacco product logs where the amount of products dispensed will be recorded and unused products collected and recorded; and c) use of biomarkers to confirm if subjects were abstinent from combustible products (e.g., CO < 6ppm, using biomarkers for volatile constituents) in the complete substitution groups. If a subject in the complete substitution group has a CO >6, a spot urine will be collected. The sample may be used to look at toxicants related to tobacco combustion which could confirm asbstinence from cigarettes.

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Compensation for Clinical Trial Phase. *S*ubjects will receive a nominal payment ($5) per clinic visit to cover transportation costs for the 7 Clinical Trial Phase visits ($40). In order to maximize abstinence in the complete substitution group, subjects will be paid for protocol compliance including staying abstinent (with biochemical confirmation) from smoking. We will use an escalating amount of money for each week of protocol compliance for all subjects (e.g., starting at $10 and for each week of compliance, payment will increase in $10 increment; if non-compliant with the protocol requirements, then the amount is reset to $10). Subjects will be told that compensation is contingent on compliance and this will be monitored via their daily diaries, product accountability, completion of forms, CO levels and potentially biomarker sample levels. If CO is elevated (CO $\geq$ 6 ppm) in the complete substitution condition, a spot urine sample will be collected for analysis of biomarkers of cigarette use (e.g. thiocyanate or anabasine). Compensation for IVR completion will start at $5 per week for week 1 of the Clinical Trial Phase and then increase $5 per week for completion of calls, if compliant. If days are missed, the payment reverts back to the $5 level. Subjects will receive $40 for the follow-up visit and $10 each for two follow-up phone calls. Total payment will be up to $585. This procedure using contingency management has been successfully used in prior biomarker studies that required total abstinence from cigarettes (see Preminary Studies). Subjects in the partial substitution group will undergo the same payment schedule and are paid for protocol compliance (attending visits, completing forms, submitting biosamples). The use of payment contingences for compliance with specific instructions for use of the snus was ruled out because of the greater likelihood of false reporting and honesty of product use is critically important. Subjects will receive free Camel Snus while participating in the study.

Measures

Subjective measures (Table 1):

The following measures comprise the TobPRAC Screening Questionnaire (developed as part of an NCI contract; N01-PC-64402):

1) Tobacco Use History and Exposure, derived from the Tobacco Use Supplement to the 2010 Current Population Survey and Population Assessment of Tobacco and Health (PATH) Survey, measures variables such as smoking amount, cigarette brand, age of initiation of smoking, number of quit attempts, duration of quit attempts, duration of smoking;
2) Demographic History inquires about age and gender, race, ethnicity, current occupation and usual occupation, and income;

3) Medical History and Current Health Status lists current diagnoses, symptoms and past health problems;
4) Concomitant Medications Questionnaire lists medications that are currently being taken;
5) Prime MD is a brief questionnaire developed for evaluation of mental disorders by primary care physicians (35);
6) Centers for Epidemiological Studies 20-item scale (CES-D) assesses current symptoms of depression (36);
7) Short Michigan Alcohol Screening Test (SMAST) Short form assesses any negative consequences from alcohol use by self report (37); and
8) Drug Abuse Screening Test (DAST) assesses negative consequences from using drugs of abuse (38).

TABLE 1 - SUBJECTIVE MEASURES

	Screen	Sample	Baseline		Intervention						F -up	Phone f-ups
WEEKS	**-93**	**-92**	**-91**	**-00**	**1**	**2**	**3**	**4**	**6**	**8**	**20**	**12 & 32**
TobPRAC Screening Questionnaire												
Tobacco Use History and Exposure; Smoking Cessation	X											
Demographics	X											
Medical History & Current Health Status	X											
CES-D	X									X		
Prime MD	X									X		
MAST	X											
DAST	X											
Biomarker Modifier Questionnaires												
Environmental & Social Influences Questionnaire			X					X		X		
NIAAA Alcohol Use Questionnaire			X					X		X		
Drug Use Questionnaire			X					X		X		
Perceived Stress Questionnaire			X			X		X		X		
Health Changes Questionnaire		X	X	X	X	X		X	X	X	X	X
Respiratory symptoms Questionnaire				X		X		X	X	X		
Concomitant Medications	X	X	X	X	X	X		X	X	X	X	X
Other Subjective Measures												
Adverse Events		X	X	X	X	X	X	X	X	X	X	X
Tobacco Daily Diary (IVR)		X	X	X	X	X	X	X	X	X		
Daily Use Summary	X	X	X	X	X	X	X	X	X	X	X	X
Fagerstrom (FTND) Test for Nicotine Dependence	X				X	X		X		X	X	
Wis. Index of Smoking Dependence Motives WISDM	X				X	X		X		X		
Stages of Change	X									X	X	
Cigarette Evaluation Scale	X	X	X	X	X	X		X	X	X	X	
Product Evaluation Scale	X	X			X	X		X	X	X	X	
Drug Effects/Liking	X	X			X	X		X	X	X		
Perceived Health Risk	X	X			X	X		X		X		
Odor and Haptics	X	X						X				
Tobacco Purchase Task	X	X				X		X		X		
PANAS			X	X		X		X		X		
MN Withdrawal Scale			X	X	X	X		X	X	X		
Questionnaire of Smoking/Snus Urges			X	X	X	X		X		X		

Interest in Switching/Quitting		X								X	X	
WEEKS	**-93**	**-92**	**-91**	**-00**	**1**	**2**	**3**	**4**	**6**	**8**	**20**	
Abbreviations: CESD= Centers for Epidemiological Studies-Depression, MAST=Michigan Alcohol Screening Test, DAST=Drug Abuse Screening Test; [1]Consumer Perception Measures from Project 3 including Oral/Haptic Scale.												

Biomarker Modifier Questionnaires

The following measures comprise Biomarker Modifier Questionnaires (measures of factors that may moderate biomarkers of exposure and effect):

1) Environmental and Social Influences Questionnaire consists of questions related to tobacco smoke exposure at home, work and socially (40);
2) NIAAA Alcohol Use Questionnaire examines rate of alcohol use, current (last month) and lifetime. This questionnaire has been adapted to include lifetime maximum use patterns (41);
3) Recreational Drug Use History assesses amount, frequency and date of last use of recreational drugs;
4) Perceived Stress Questionnaire is a 14-item form measuring the degree to which life situations are appraised as stressful (43);
5) Health Changes Questionnaire assesses any new health problems since their last clinic visit;
6) Respiratory Symptoms Questionnaire is used to rate cough, phlegm production, shortness of breath and other respiratory symptoms on a scale ranging from 0 = none up to 10 = severe with a total respiratory score determined by adding the scores of each of these items.

Subjective outcome measures:

1) Interactive Voice Response system will be used on a daily basis to record amount of product use, cigarette and other nicotine containing products and tobacco use status will be reviewed and recorded at each clinic visit;
2) Fagerstrom Test for Nicotine Dependence (FTND) (44);
3) Wisconsin Index of Smoking Dependence Motives is a multidimensional measure of dependence based on theoretically grounded motives for drug use and intended to reflect mechanisms underlying dependence and has been found to be related to smoking heaviness, DSM-IV symptoms of depression and relapse (45);
4) Minnesota Nicotine Withdrawal Scale (46);
5) Questionnaire of Smoking Urges, a 32-item questionnaire on smoking urges, assesses a multidimensional conceptualization of craving to smoke (47);
6) Adverse Events Scale assesses the nature, severity, duration, action taken, and outcome of adverse events related to tobacco product use;
7) Perceived Health Risks Assessment asks subjects to rate their perception of their usual tobacco product against the study product to which they were randomly assigned;
8) Product Evaluation Scale is a 7-point Likert-type scale that is modified from the Cigarette Evaluation Scale (48). In addition, items from a scale that was used to evaluate various medicinal nicotine products is incorporated in the Product Evaluation Scale (49). This scale has been used in a number of our studies. Cigarette Evaluation Scale will be used at baseline;
9) Drug Effects/Liking Questionnaire (50);
10) Stages of Change (51);
11) Consumer Product Perception. This measure will be developed by Project 3 of this U19 and include cognitive and affective measures and Odor/Haptics; and
12) Tobacco Purchase Task. Participants will be asked “How much would you be willing to pay for one tin of Camel Snus?” As a reference point, participants are also asked to report how

much they usually pay for one pack of cigarettes. The ratio of these prices can give an indication of the relative value of Camel Snus, and would allow comparison across the study sites, where cigarette prices vary. Subjects will also be provided the Tobacco Purchase Task developed, which will be used to generate demand curves by asking participants to report the number of units of a tobacco product that they would consume in a day if the units cost various amounts of money. This procedure has been used to study demand for cigarettes (52-53).

13) Interest in Switching or Quitting assesses interest in switching from cigarettes to the Camel Snus if it were not for the study; impact on interest in quitting tobacco use; and interest in paying for the product.

A panel of biomarkers has been selected to represent exposures to different toxicants and different disease risk factors. Samples will be analyzed for some or all of the following biomarkers and will be sent to a Biobank with de-identified information. Please see Table 2 for list of biomarkers.

1. Carcinogen biomarkers of exposure and effect and cardiovascular risk indicators were chosen because they have shown reasonable laboratory reproducibility, have clear differences in levels between smokers and nonsmokers and/or decrease upon tobacco cessation (33-34).
2. Cardiovascular biomarkers were selected because they are associated with different pathogenesis for disease and have also shown reasonable laboratory reproducibility.
3. Nicotine exposure and metabolite ratio (NMR) and the total nicotine equivalents will be determined. The latter accounts for 73-96% of the nicotine dose and are a useful measure of daily nicotine exposure. The NMR is an indicator of CYP2A6 enzyme activity and is the ratio between two nicotine metabolites, cotinine and trans-3'-hydroxycontinine (3-HC).

TABLE 2 - BIOMARKER MEASURES

[1]***Biomarkers for Carcinogen Exposure***											
	Screen	**Sample**	**Baseline**		**Intervention**						**20 F-up[4]**
WEEKS	**-93**	**-92**	**-91**	**-0**	**1**	**2**	**3**	**4**	**6**	**8**	**20**
Total NNAL			X	X		X		X		X	
Total NNN			X	X		X		X		X	
Phenanthrene Tetroal			X	X		X		X		X	
Mercapturic acids of acrolein, benzene, 1,3-butadiene, crotonaldehyde, and ethylene oxide			X	X		X		X		X	
PGEM			X	X		X		X		X	
8-epi-$PGF_{2\alpha}$			X	X		X		X		X	
[1]***Nicotine Exposure and Metabolite Ratio***											
Total nicotine equivalents (Serum/Urine)			X	X				X		X	X
Nicotine metabolite ratio (serum)				X							
C-Reactive protein*				X				X		X	
Hematology				X				X		X	
Other Biomarkers											
Alveolar carbon monoxide (CO)[2]	X	X	X	X	X	X	X	X	X	X	
Oxygen saturation	X	X	X	X		X	X	X	X	X	

Expectorated SLT samples/Cig Butts and Filters				[2]X				X			
Vital signs and weight	X	X	X	X		X	X	X	X	X	X
Buccal cells				X				X		X	
Urine pregnancy screen	X										
[3]Biobank Samples											
Urine			X	X		[3]X		[3]X	X	X	X
Spot urine (if appropriate)					X	X	X	X	X	X	X
Blood				X		X		X		X	X
Genetic analysis[5]											
WEEKS	**-93**	**-92**	**-91**	**-0**	**1**	**2**	**3**	**4**	**6**	**8**	**20**

[1]Justification for Biomarkers in Core B and Hatsukami et al., (2005); [2]Cigarette butts only
[3]Samples collected at weeks 2 and 4 will be stored for potential analyses in the future as needed or funded.
[4]Only week 20 at follow-up will be a clinic visit, week 12 and 32 are telephone; [5]Samples will be stored for future funding for analysis for genetic predisposition for tobacco toxicant metabolism, behavior and harm. Abbreviations: Total NNAL: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; Total NNN: *N'*-Nitrosonornicotine and its glucuronides; PheT: *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; Mercapturic acids: *S*-Phenylmercapturic acid; Monohydroxybutylmercapturic acid; 3-Hydroxypropylmercapturic acid; 4-Hydroxybut-2-ylmercapturic acid; 2-Hydroxyethylmercapturic acid; PGE-M: 11-α-Hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid; 8-epi-$PGF_{2\alpha}$:9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid. NOTE: Minor tobacco alkaloids (nornicotine, anabasine, anatabine, myosamine and cotinine) will be analyzed in 50 complete substitution subjects for the analysis proposed by Project 1.

Other biomarkers will include:

1. Exhaled carbon monoxide (CO) and oxygen saturation as indicators of the extent to which red blood cells may be carrying their usual load of oxygen.
2. Biobank: Other tobacco-related biomarkers for assessment in blood, urine, or buccal cells may be determined to be necessary or may be developed over time and these additional assays will be completed as appropriate. Also, in the future, samples may be analyzed for genetic predisposition for tobacco toxicant metabolism, behavior and harm.

Product constituent testing:

Pouches of used smokeless tobacco product will be obtained for chemical constituent analysis. Prior to the clinic visit at week 4 subjects will be asked to collect all used samples during the 24 hours before the visit. These samples will be placed in a small cooler and brought to the clinic visit. In addition, five unused oral tobacco pouches from their tin will be collected. Both these samples will be frozen. These samples will be shipped on dry ice to the CDC when samples from all the visits have been collected. At the CDC, the levels of selected chemical constituents in the unused and used pouch will be analyzed. These chemical constituents include nicotine, tobacco specific nitrosamine (NNK, NNN), BaP and catechols. Solanesol in cigarette filters will also be tested by CDC, which is a measure of tobacco smoke exposure (see 30, 31 for description of analysis conducted at CDC).

Product intervention dose:

The dose of unprotonated nicotine for Camel Snus Frost is 3.58, Camel Snus Mellow is 3.36, Camel snus Robust is 5.09 and Camel Snus Winterchill is 4.59 and our prior PK study (unpublished) showed that these doses led to levels that were about equivalent to or slightly higher than 4 mg medicinal nicotine lozenge. We will initiate use with the highest dose and if side effects are experienced, then we will assign the product with the lower dose. In our current study, the majority of smokers are able to tolerate the higher doses (95%; 21 out of 22 smokers)

and choose Winterchill (82%, 18 out of 22 smokers). The levels of exposure to toxicants (e.g., carcinogens) are lower than observed from cigarettes (2).

6.5 Potential Risks of Study Participation:

The potential risks for subjects are minimal. Subjects' medical history will be reviewed at orientation prior to entry into the study. Subjects will be under medical supervision throughout their participation in the study and adverse symptoms will be recorded at each clinic visit and monitored by the PIs and study medical personnel. We will also be available 24 hours a day for emergency calls.

The major side effects associated with Camel Snus are nausea, dizziness and lightheadedness, mouth sores, gastrointestinal distress, headache, hiccups, dry mouth and sore throat. Nicotine is toxic to the fetus and pregnant women are excluded from participation.

The oral tobacco product administered to subjects is currently marketed and sold over-the-counter. All subjects are current regular smokers and thereby exposed to nicotine and tobacco toxicants. Oral tobacco products may be linked with an increased risk for oral and pancreatic cancer, although compared to cigarettes, the risk is dramatically less. The reduction in risk is largely due to delivery of lower number and sometimes levels of tobacco toxicants from smokeless tobacco to the user simply because the tobacco is not burned, which yields thousands of combustion products. However, as this study will examine, some users may maintain their baseline smoking rate while using oral tobacco thereby may increase their exposure to nicotine and perhaps some tobacco related toxicants. We will minimize this risk by seeing the subjects on a weekly and then bi-weekly basis to assess increased tobacco use. In addition, all subjects will be strongly advised to quit all tobacco use at the end of the study and will be provided cessation materials.

There is also the chance of continued use of the products after the study has ended; however, cessation of all tobacco products will be recommended to the subjects. If at any time the subject wants to quit smoking or use of all tobacco products, this decision will be encouraged and supported. Subjects will be provided with a treatment manual and referral to different treatments.

Other risks include blood draws and a trained laboratory technician or a registered nurse will obtain blood samples. Otherwise, the physiological and subjective measures will be noninvasive and should present no psychological or medical risk to the subject.

Some questionnaires may be of a sensitive nature assessing subjects' drug and alcohol use or psychiatric symptoms. Subjects will be told that they may refuse to answer, however, refusal may effect continued participation in the study.

7.0 Statistical Analysis

This study has three primary aims: to determine the effect of instructions for use and the amount of substitution on biomarkers of exposure and patterns of oral tobacco use (primary endpoint at 4 weeks, but also assessed at 8 weeks), to determine when stabilization of product use occurs by examining the time effects for subjective, biological, topographic and behavioral responses (analysis for 2, 4, 6 and 8 weeks) and to determine important moderators for product use and exposure. Secondary aims include developing innovative measures to assess levels of exposure from smokeless tobacco and cigarettes.

Demographic and smoking history variables of those who entered the sampling phase and the trial phase will be summarized and compared to national survey data for the general population of smokers and smokers most interested in using oral tobacco products (e.g., 1). Baseline covariates will be summarized by treatment group to check that groups are balanced after randomization. Continuous measures will be summarized by mean (SD), median (range)

or other summary measures. Categorical covariates will be summarized by contingency tables. Numerical variables measured more than once at the baseline assessments will be assessed for reproducibility using the intra-class correlation coefficient and the average will be used for the baseline variable if adequate reproducibility is observed.

The primary outcome measures (tobacco use, biomarkers, subjective measures) will be assessed up to five times for each individual. The analysis of our primary aims will use linear mixed models to account for potential intra-subject correlation due to repeated measurements from a single individual. Linear mixed models are sensitive to miss-specification of the within-subject correlation structure. We will use exploratory graphical methods to identify the appropriate within-subject correlation structure. Residual plots and other diagnostic methods will be used to check other model assumptions.

Hypothesis 1 will be analyzed by fitting a linear mixed model for each of the outcome measures (using week 6 as the primary endpoint): total NNAL, NNN, phenanthrene tetarol, mercapturic acids, PGEM, 8-epi-$PGF_{2\alpha}$, Total nicotine equivalents; and cardiovascular risk factors.. The mean model will include an effect for time, group and a time by group interaction. Specific hypotheses will be tested by testing the significance of the appropriate contrast. A secondary analysis will be completed adjusting for important factors such as environmental toxicant exposures, nutrition, stress and health to determine the effect their inclusion has on the association between group and the outcomes. Finally, we will complete an exploratory analysis that includes a site by group interaction to determine if the group effect changes by location.

Hypothesis 2 will be analyzed by fitting separate linear mixed models for each group using biomarkers, snus topography, cigarettes and other subjective responses as the outcome. We will test for significant time effects and present plots of the outcomes to visually represent the time effects. Stabilization will be considered to have occurred when the variance between time-points does not exceed the coefficient of variation of the measures as assessed in the usual brand smoking group.

Hypothesis 3 will be analyzed using a linear mixed effects models with a mean model that includes time, group and potential moderators (e.g., use patterns, age, sex, dependence, subjective responses, etc.). Interactions between group and the potential moderators will be included in the model and tested to determine if the potential moderator modifies the effect of group on the outcome (total nicotine equivalents, total NNAL).

Hypothesis 4 will be evaluated using scatter plots and other visual methods. Associations will be summarized by Pearson's or Spearman's correlation coefficient, as appropriate, and formal inference will be completed using linear regression.

Compliance to the assigned treatment is a concern in this study. Our primary analysis will follow the intent-to-treat principle, where subjects are analyzed in the group they were randomized to regardless of compliance, to preserve the integrity of a randomized study. In addition, we will summarize compliance by treatment group and evaluate the correlation between compliance and baseline covariates to identify potential predictors of treatment compliance. Finally, we will complete a secondary analysis that accounts for non-compliance. A standard analysis adjusting for compliance in a regression setting is known to be susceptible to selection bias and, therefore, our secondary analysis will be completed using a principle stratification framework (56).

<u>Other Analyses of Interest</u>

Hypothesis: Those subjects who rate the products positively on the Product Evaluation Scale, Drug Effects/Liking Questionnaire and Perceived Health Risks during the sampling phase and extent of willingness to pay for the product are more likely to use more products during sampling, remain in the study and use products during the clinical trial phase.

Hypothesis: Most subjects (controls) will choose the *ad libitum* partial substitution method for tobacco use the most and very few smokers will choose the complete substitution condition. The rate at which subjects choose the various methods for use will be summarized by the sample proportion and confidence intervals.

Interaction of components tested across all projects will be conducted under Core A

Sample-size determination.

A sample of size 138 per group (690 in the five groups) would be required to assure an 80% chance of demonstrating that complete substitution leads to higher reduction in NNAL levels compared to the partial substitution after controlling for baseline NNAL levels (assuming a correlation of 0.6 between baseline and follow-up NNAL scores, and an effect size of low to medium) and accounting for drop-out rates and an abstinence rate of 40 to 50%. See Human Subjects section for details.

Randomization: Stratified randomization will be generated by the Biostatistics and Informatics Core of this grant.

8.0 ADMINISTRATIVE CONSIDERATIONS

8.1 Conduct of the Trial

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP), the Declaration of Helsinki, and the appropriate regulatory requirement(s).

The University Of Minnesota IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/EC or Campus Administrator approval has been obtained. The protocol, informed consent, written information given to the patients, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

8.2 Data Management

Quality control and adherence to protocol procedures:

Standard operating procedures will ensure that all study sites are following the same procedures. Face-to face training for research personnel will occur prior to the study, where the protocol and procedures will be carefully described. Case report books will be provided for the different sites to maximize parallel data recording. Each visit will have a checklist of all the measures that need to be taken and the order by which these measures are administered. As another measure to ensure data collection integrity, an experienced study monitor will make a visit to clinical sites after they have enrolled their first several subjects to make sure that the protocol is being followed carefully and all the data are being collected properly. Another onsite visit will occur after the initiation of the study. Regular telephone conference calls will occur among the research coordinators to provide updates on study progress, trouble shoot problems and to make sure that all protocol procedures are followed. On a once a month basis, the investigators will participate in this conference call. During these conference calls, the number of subjects enrolled, the data collection process, the results from data monitoring and other issues of concern will be discussed.

Urine and blood biosamples and pre- and post-use smokeless tobacco (ST) collections will be given individualized bar codes for each subject (See Biomarker Labeling SOP). Each site will receive barcoded labels which have been packaged at the research coordinating site (University of Minnesota). Most of the subjective measures will be administered via Qualtrics, a secured website for the subject to enter the date in electronic forms. Forms will include programming features to ensure valid data (i.e., input masks, validation criteria, skipout logic) and will be exported to files that are de-identified of any personal health identifiers.

All other data not captured directly in Qualtrics at the other sites (e.g., pencil-and-paper forms) will be sent to the coordinating site (with photocopies of originals maintained at the other sites). A data monitor will be available at the research coordinating site and irregularities (e.g., wrong date on the forms, inconsistent data) or missing data will be flagged by the monitor and sent to the site for comment or correction to ensure the integrity of the data.

All de-identified biosamples will be sent to the University of Minnesota on a quarterly basis. Lists of subject number and biosamples sent will be recorded by each site and when the samples are received, the researcher at the coordinating site will note each subject number and biosample that was received. The samples-received list will be posted on a secure website so that each site can check it against their own lists.

Data Identification:

Human subject consent forms will indicate corresponding subject ID and will not be stored with the data. Paper and pencil subjective forms, and other Case Report Forms (CRF) will be identified with subject number and initials, and visit number and date will be entered onto appropriate forms. Data entered into the database will be de-identified, with only subject number as a link. Biomarker samples will be labeled with a scanner barcode indicating study site, subject number, visit number, specimen type (e.g., urine, serum, saliva) and intended assay or biobank samples.

All records will be confidential. All shared date will be de-identified. A Certificate of Confidentiality from NIH will be obtained to protect subject data from subpoena. A study monitor from the University of Minnesota will review each site's records to ensure all the study records are accurate and that all consents have been signed, thereby having access to names of individuals at different sites. However, no record of this identifying information will leave the premises. All data from the subjects at the different sites will be on a secure web-site or sent to the University of Minnesota, and all information will be de-identified. All biosamples sent to the University of Minnesota will also be de-identified.

Randomization Procedures:

Randomization will be stratified by location and number of cigarettes smoked per day (≤ 18/day vs. > 18/day, the expected median number of cigarettes smoked per day) to assure balance across treatment groups. Treatment groups will be checked for balance on other factors post randomization and any imbalances will be accounted for in our analysis as described in the statistical analysis section.

Subjects who have completed the Sampling Phase and are interested and eligible (use of ≥14 sachets and used most days) will be entered into the clinical trial and randomized to one of 5 arms. Subjects will be randomly assigned to the groups in an unblended fashion using a blocked random assignment list generated by the Biostatistics and Informatics Core. Each study site will receive a copy of the Randomization List for their study site. Subjects will be assigned sequentially to the product associated with the next open randomization number. This randomization number will be entered into the CRF.

Study Tobacco Product and Accountability:

The dose of Camel Snus (Winterchill and Robust) is about 2.82 mg of free-nicotine per portion and our prior PK study (unpublished) showed that the dose is likely to lead to levels that were no higher than 4 mg medicinal nicotine lozenge. If these doses are too high, subjects will be switched to lower nicotine level Camel Snus products (Frost and Mellow). In the experimental condition that involves specific amounts of use, we based the dosing schedule on prior study results that showed that smokers who smoke 20 cigarettes a day on average use 7-8 pouches of Camel Snus and a study conducted by Dr. Thomas Eissenberg who found that those who smoke >20 cigarettes a day use about 12 packets per day. The levels of exposure to toxicants (e.g., carcinogens) are lower than observed from cigarettes (Kotlyar et al.).

Record of study product purchased will be available at the study site showing number of tins purchased, purchase site, and purchase price. Study product accountability will be also documented in the CRF under the "Product Accountability" form denoting the lot number for the tin, the number of tins of Camel Snus dispensed, number of unused tins returned, and any discrepancy between dispensed, and reportedly used product on the daily diary.

Biobank:

A separate consent form will be used to obtain permission from subjects to allow de-identified biosamples to be stored in a biobank for future analyses of biomarkers or genotyping. Investigators at each site will only have access to identifying subject information of their subjects. This information will be kept locked in a secure area. Neither the other sites nor the main coordinating site will have access to this information. All biobank samples will be in the PL control and collaborating researchers will submit an application to be reviewed and assessed by PL prior to sample sharing.

8.3 Data and Safety Monitoring Plan

The study coordinator and the research nurse will be responsible for the daily oversight of subject safety. At the University of Minnesota site, Drs. Dorothy Hatsukami and the Medical Director, Dr. Sharon Allen, will meet weekly with the study staff to review patient's progress and their experiences with the tobacco products, including any adverse events. Entrance criteria will be reviewed following screening. Medical history will be reviewed for any contraindications for the treatment products and vital signs checked on a weekly basis. Patients will be under medical supervision while in the study and seen on an ongoing basis by our research staff who will assess adverse events and make appropriate referrals to the physician. Similar procedures will be instituted at the other institutions. Any adverse symptoms will be discussed over the telephone on the weekly calls across the sites. Urgent issues can be dealt with more immediately.

Drs. Hatsukami and Shields will conduct internal monitoring of subject safety across all sites. Any medical issues that occur at the three other sites will be conveyed to them. These issues will be recorded and tracked. In addition, a Data and Safety Monitoring Board, which will be comprised of experts in the areas of smoking tobacco products, tobacco addiction and clinical trials, will be convened. The Board will include Patrick Nan-Sinkam MD, Associate Professor of Internal Medicine and Co-Director Research Programs in the Division of Pulmonary Allergy, Critical Care and Sleep Medicine at Ohio State University; Michael Kotlyar, PharmD, Associate Professor of Experimental and Clinical Pharmacology at the University of Minnesota; Scott Leischow, PhD, Professor in the UA College of Medicine; and Haitao Chu, PhD, Associate Professor, Division of Biostatistics, School of Public Health.

The Data and Safety Monitoring Board will begin by reviewing the protocol and establishing guidelines for data and safety monitoring. This will include developing standard procedures for day-to-day monitoring by the internal monitors, investigators and study staff. This Board will

meet at regular intervals (at least once a year) to evaluate the progress of the trial, review data quality, patient recruitment, study retention, and examine other factors that may affect study outcome. They will also review the participant's ability to achieve the study requirements and the rates of adverse events to determine whether there has been any change in participant risk. Their review will ensure that subject risk does not outweigh the study benefits. A brief report will be generated from each of these meetings for the study record and forwarded to the each of the study site's Institutional Review Boards (IRB).

The DSMB will be available to convene outside of the regular meetings, if necessary, if concerns should arise regarding a particular subject, or any troublesome trends in the patient experiences. They will make appropriate recommendations for changes in protocol, if needed.

The University of Minnesota NCI designated Cancer Center also has a Cancer Protocol Review Committee that meets on a monthly basis to review all cancer-related protocols. This study will also be subjected to review by this committee. Reports to this committee are submitted on a yearly basis, and for some projects, on a quarterly basis. Reports from all sites will be submitted.

All drug related adverse events of a non-serious nature will be reported each institution's IRB on at the time of renewal. Serious adverse events related to the study product or procedures will be reported by telephone to the IRB within the 3 days of our receipt of information regarding the event and written reports will be submitted within 10 days. The Data and Safety Monitoring Board will review all serious or unexpected adverse events and provide recommendations.

We will inform NIH of any significant action taken as a result of the Data and Monitoring Board's findings. We will inform the subjects of any changes in risk.

8.4 Event Reporting to IRB and Cancer Center's Data and Safety Monitoring Council (DSMC)

Safety concerns for this project are expected to be minimal and include breach of confidentiality; issues related to coercion; effects of nicotine withdrawal; effects of returning to regular smoking; and effects associated with Camel Snus. The expected safety concerns will be addressed at IRB annual review unless it is appropriate to file a UPIRTSO immediately.

Any events meeting an unexpected, serious adverse event defined as reportable (such as hospitalization) on the IRB's website at http://www.research.umn.edu/irb/ae/. The DSMC will be copied on all reports to the IRB.

In addition, to be in compliance with local and federal regulations the following events/problems will be reported to the IRB and DSMC within the 10 working day time frame:

- Any serious event (including on-site and off-site adverse events, injuries, side effects, deaths or other problems) which in the opinion of the local researcher was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures
- Any serious accidental or unintentional change to the IRB-approved protocol that involves risk or has the potential to recur;
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject;
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research;
- Any breach in confidentiality that may involve risk to the subject or others;

- Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the research staff.

Adverse Events

An Adverse Event (AE) is any untoward occurrence in a subject administered a study product and it may or may not have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding) and symptom or disease temporally associated with the use of an oral tobacco product, whether or not considered related to the product. AEs include any illness, sign, symptom, or clinically significant laboratory test abnormality that has appeared or worsened during the course of the clinical trial, regardless of causal relationship to the product(s) under study. Each AE will be evaluated to determine severity (mild, moderate, severe); duration; relationship to study product; action taken, if any; and whether it meets criteria to be considered a Serious Adverse Event.

Adverse events will be assessed at each visit and compared to baseline rates of occurrence. All adverse events of a severe or serious nature will be presented to the PI and medical oversight at the study site and the coordinating site will be informed of the event.

Serious Adverse Events

Serious Adverse Event (SAE) is an adverse event occurring at any dose that results in either: 1) Death; 2) "Life-threatening" event; 3) Persistent or significant disability/incapacity; 4) Requires or prolongs hospitalization; 5) Congenital anomaly or birth defect; 6) Cancer; 7) An important medical event which may jeopardize the patient and may require intervention to prevent a serious outcome; or 8) A medically significant/important event based on the Investigator's assessment. All Pertinent information for completing serious adverse events CRF will be obtained including: Subject Number, demographics, diagnosis (if known) or symptom, date of onset, maximum intensity, outcome, date of resolution or death, action taken with study drug, withdrawal from study, relationship to study drug, possible cause of serious event other than study drug, relevant medical conditions or risk factors, current medications or other relevant details or assessments such as laboratory data and/or test results. Details of all study product taken until the onset of SAE should be provided. The association or relationship of the study product with a Serious Adverse Event will be determined by the clinical investigator.

Reporting Requirements

A serious event (SAE) must reported to the coordinating site (UMN) if it is both unexpected and associated with the use of the study product within 24 hours of acquiring the information. The coordinating site will notify the FDA, IRB and all investigators of the event, in writing, within 10 working days after initial receipt of the information. Since this study is not conducted under an IND, telephone reports within 24 hours will not be required.

Any SAE occurring after the patient has signed the informed consent and until 4 weeks after the patient has stopped study participation must be reported. SAEs occurring more than 4 weeks after study discontinuation need only be reported if a relationship to ST use is suspected.

COLLABORATING SITES:

The sites include University of Minnesota (Dorothy Hatsukami), Ohio State University (Peter Shields) and Roswell Park Cancer Institute in Buffalo, NY (Richard O'Connor). Dorothy Hatsukami will lead and oversee the project. Each site will have a Project Leader and Co-leader, Project Coordinator and study assistant. All sites will obtain IRB approval from their institutions with reference to the main institution (University of Minnesota), which will be collecting all data

and receiving biosamples. All personnel will be trained on study procedures, human protection issues and regulatory requirements.

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