

eSupplement 1

This eSupplement 1 has been provided by the authors to give readers additional information about their work.

eSupplement 1 to: Hatsukami DK, Luo X, Jensen JA et al. **Effect of immediate vs. gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: a randomized clinical trial**

Effect of immediate vs. gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: a randomized clinical trial

Dorothy K. Hatsukami, PhD¹; Xianghua Luo, PhD¹; Joni A. Jensen, MPH¹; Mustafa al'Absi, PhD²; Sharon S. Allen, MD³; Steven G. Carmella, BA¹; Menglan Chen, MS¹; Paul M. Cinciripini, PhD⁴; Rachel Denlinger-Apte, MPH⁵; David J. Drobes, PhD⁶; Joseph S. Koopmeiners, PhD¹; Tonya Lane, MEd¹; Chap T. Le, PhD¹; Scott Leischow, PhD⁷; Kai Luo, PhD¹; F. Joseph McClemon, PhD⁸; Sharon E. Murphy, PhD¹; Viviana Paiano, MS¹; Jason D. Robinson, PhD⁴; Herbert Severson, PhD⁹; Christopher Sipe, MS¹; Andrew A. Strasser, PhD¹⁰; Lori G. Strayer, MPH¹; Mei Kuen Tang, BS¹; Ryan Vandrey, PhD¹¹; Stephen S. Hecht, PhD^{1*}; Neal L. Benowitz, MD^{12*}; Eric C. Donny, PhD^{13*}

1 University of Minnesota, Masonic Cancer Center, Minneapolis, Minnesota

2 University of Minnesota Medical School, Behavioral Medicine Laboratories, Duluth, Minnesota

3 University of Minnesota Medical School, Family Medicine and Community Health, Minneapolis, Minnesota

4 University of Texas MD Anderson Cancer Center, Department of Behavioral Science, Houston, Texas

5 Brown University, Department of Behavioral and Social Sciences, Providence, Rhode Island

6 Moffitt Cancer Center, Department of Health Outcomes and Behavior, Tampa, Florida

7 Mayo Clinic, Health Sciences Research, Scottsdale, Arizona

8 Duke University, Department of Psychiatry and Behavioral Sciences, Durham, North Carolina

9 Oregon Research Institute, Eugene, Oregon

10 University of Pennsylvania, Department of Psychiatry, Philadelphia, Philadelphia

11 Johns Hopkins University, Department of Psychiatry and Behavioral Sciences, Baltimore, Maryland

12 University of California, Department of Medicine, San Francisco, California

13 Wake Forest School of Medicine, Department of Physiology and Pharmacology, Winston-Salem, North Carolina

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Study Protocol (Investigational Plan)

Protocol for Hatsukami, Luo, Jensen, et al., Effect of immediate vs. gradual reduction in nicotine content of cigarettes on biomarkers of smoke exposure: a randomized clinical trial

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AIMS, RATIONALE AND BACKGROUND

Specific Aims and Hypotheses:

The main goal of this project is to compare two different approaches to reducing levels of nicotine in cigarettes: an immediate reduction in nicotine content in cigarettes to non-addictive levels or a gradual reduction in nicotine content in cigarettes to non-addictive levels. These two approaches will then be contrasted to a group that continues to smoke cigarettes with nicotine content similar to conventional cigarettes. Towards these goals, the follow specific aims will be addressed.

Specific aim 1: To compare product biomarkers of exposure between smokers assigned to a) gradual reduction in reduced nicotine content (RNC) cigarettes; b) Immediate reduction to very low nicotine content (VLNC) cigarettes or c) experimental cigarettes with a conventional nicotine yield. **Hypothesis:** Immediate reduction to VLNC cigarettes will lead to lower levels of biomarkers of exposure (AUC) than a gradual reduction in RNC cigarettes; both experimental conditions will lead to lower exposure levels (AUC) than the conventional nicotine yield condition. This lower level of exposure in the immediate reduction approach will be related to reduced number of cigarettes smoked and greater rate of abstinence compared to the gradual reduction approach. Both these approaches will be associated with fewer cigarettes smoked and greater abstinence than the conventional nicotine yield condition.

Specific aim 2: To compare subjective (e.g., withdrawal [MNWS], dependence [FTND and WISDM], product liking [Drug Effects/Liking Scale] and satisfaction [Cigarette Evaluation Scale/CES]) and other behavioral responses (e.g. compliance and retention) for the two experimental cigarette conditions and compared to conventional nicotine yield condition. **Hypothesis:** Time course of subjective and other behavioral responses will differ across the two experimental cigarette conditions and control condition. For example, no real change will occur with gradual RNC cigarette group until substantially lower levels of nicotine (< 0.2) are introduced (i.e. at later follow-up time points), while the immediate VLNC group will experience a more immediate and acute change (i.e. at earlier follow-up time points) in subjective response (e.g., increase in withdrawal symptoms, less dependence, less cigarette satisfaction) and behavior (e.g., greater non-compliance, more dropouts) but will decrease over time. Control group will show no change in responses.

Specific aim 3: To determine important moderators (i.e., nicotine metabolite ratio, nicotine dependence, gender and race) of product use and exposure and study retention, particularly with VLNC cigarettes. **Hypothesis:** Greater intensity of VLNC cigarette use and study retention will be related to being female, being African American, having a higher degree of dependence and having faster nicotine metabolism.

The outcomes from this study will provide information on different approaches to reducing levels of nicotine in cigarettes and will determine the approach with the most optimal outcomes taking into account the balance between overall risk reduction (possibly maximized by abrupt switching) and compliance and acceptability (possibly maximized by gradual reduction of RNC cigarettes).

Background Information:

Rationale:

Over 44 million people in the United States smoke (1) and about 1.2 billion world-wide smoke cigarettes (2). Developing countries are particularly vulnerable for increases in tobacco use. With 440,000 deaths per year in the US and 6 million per year world-wide with speculations of 7 million deaths per year if current trends in smoking continue through 2020 (2), it is critical to have strong tobacco control policies in place to minimize the casualties from tobacco use.

According to Orleans and Slade (3) and Giovino (4), there are four main targets for tobacco control: the Agent, the Vector, the Host and Environment. The majority of recent tobacco control efforts have been aimed at the Host (tobacco prevention and cessation programs), the Environment (policies such as smoking bans, increased taxes, anti-smoking media campaigns, advertisement bans, pictorial warning labels) and the Vector (tobacco law suits). Relatively little attention has been focused on the Agent (the tobacco product). Altering tobacco products in ways to reduce mortality and morbidity that complement current tobacco control measures may be an important next step in our tobacco control efforts.

The Family Smoking Prevention and Tobacco Control Act (FSPTCA) passed in 2009 provides the FDA with the authority to regulate tobacco products. One of the provisions in this legislative act empowers the FDA to reduce harmful constituents in tobacco products, including nicotine as long as the nicotine levels are not reduced to zero. Reducing toxicants in cigarettes to established performance levels, as proposed by the World Health Organization (5), is a worthy endeavor. However, the only way that a combusted product is likely to result in reduction in harm is if the product is rendered non-addictive (6, 7). Such a measure potentially can reduce individuals experimenting with smoking from becoming addicted to tobacco and would help current smokers quit smoking. Although the proposal to reduce nicotine in cigarettes has been met with skepticism because of concerns over compensatory smoking behavior (8) and the emergence of a black market (9), this policy measure was considered to be technically feasible by the American Medical Association and the British Medical Association (6) and by tobacco control researchers, policymakers and governmental officials who were convened in a meeting on nicotine regulation (10). The public health impact of this approach has been estimated to be equivalent to the impact of sanitation efforts in reducing death and disease, despite any mortality increases due to compensatory smoking or the emergence of a black market; and the prevalence of smoking was projected to decline from 23% to 5% (11).

Reducing nicotine content in cigarettes:

About 20 years ago, a proposal for a nationwide gradual reduction of nicotine levels in cigarettes was described as a means to decrease the initiation of smoking (12). Although this approach has been conceptually supported by researchers and medical organizations as a potential policy measure, a number of research questions, such as the impact of reduced nicotine cigarettes on smoking behavior, toxicant exposure and cessation, need to be addressed prior to its full endorsement (6). One critical research gap that requires attention is determining the most optimal approach for nicotine reduction—gradual reduction of reduced nicotine content (RNC) cigarettes as described in the Benowitz and Henningfield article (12) vs. immediate reduction to very low nicotine content (VLNC) cigarettes (10). A study conducted by Benowitz et al. (13) examining the gradual nicotine reduction approach (5 different RNC cigarettes with nicotine yields ranging from 0.8 to 0.1 mg nicotine yield, each smoked for one week) found evidence of minimal compensatory smoking, decreases in exposure to tobacco toxicants and in cotinine, minimal although some withdrawal symptoms, reduction in dependence and an abstinence rate of 25%. In another similarly designed study using filter ventilated cigarettes, no decrease in cotinine or tobacco toxicant exposure was seen until smokers begin smoking cigarettes at 0.2 and/or 0.1 mg nicotine yield cigarettes, and at the end of the trial, an abstinence rate of 10% was observed (14). In a more recent study, Benowitz and colleagues had smokers smoke RNC cigarettes, switching to cigarettes reduced in nicotine content every month (15). The results were similar to the prior studies that demonstrated only modest compensatory smoking at higher RNC cigarettes, no increased toxicant exposure, a significant decrease in cotinine, minimal manifestation of withdrawal symptoms, and a reduction in cigarettes smoked at the 0.1 mg nicotine yield dose. In a study in which smokers interested in cessation were asked to switch immediately to a VLNC cigarette (0.05mg), a low nicotine cigarette (0.3 mg) or oral medicinal nicotine product for 6 weeks (16), smokers experienced increased and therefore, compensatory smoking with the 0.3 mg nicotine yield cigarette, but a gradual decrease in smoking with the 0.05 mg cigarette. The 0.05 mg nicotine cigarette group also experienced minimal withdrawal after switching from usual brand cigarettes to these cigarettes, a reduction in dependence, a reduction in toxicant exposure and a higher cessation rate (36% with 0.05 mg cigarettes, 14% with 0.3 mg cigarettes, and 20% with oral medicinal nicotine). Both approaches appear to be feasible, but to date, no direct comparisons have been made between these approaches. To address this question, this study proposes to randomize smokers to three different experimental conditions: 1) very low nicotine content (VLNC) cigarettes with an approximate 0.03 mg nicotine yield for menthol and non-menthol cigarettes (actual dose to be determined by Project 1; n=500); 2) gradual reduction in nicotine content cigarettes (non-menthol cigarette nicotine yields: 0.8 mg, 0.7 mg, 0.26 mg, 0.12 mg and 0.03 mg yield cigarettes; menthol cigarette nicotine yields: 0.8 mg, 0.6 mg, 0.26 mg, 0.12 mg, 0.03 mg, each RNC cigarette smoked for a period of 4 weeks, n=500); and 3) experimental cigarettes with nicotine yields similar to usual brand cigarettes (0.8 nicotine yield) as a control group (n=250). Over the 20-week experimental phase, participants will be assessed for pattern of tobacco use, biomarkers of exposure and effect, subjective responses (e.g., satisfaction, craving withdrawal symptoms) and behavioral responses (compliance, dropping-out). At the end of the experimental phase, they will be followed for another 4 weeks to assess abstinence from all tobacco products and smoking rate.

Individual differences in response to denicotinized or VLNC cigarettes:

The response to RNC cigarettes and approaches for nicotine reduction may depend on individual characteristics of the smoker (10). To date, only a few studies have provided some insights of potential predictors of responses to RNC cigarettes. For example, in a study examining individual differences in responses to nicotine containing vs. VLNC cigarettes, smokers who were more dependent rated these two types of cigarettes more similar than less dependent smokers (17). The authors speculated that because of more intensive pairing between smoking related sensory cues and pharmacological effects of nicotine in the heavily dependent group, sensory aspects of smoking is a greater secondary reinforcer in heavier smokers. Alternatively, heavy smoking may lead to greater tolerance, resulting in less incremental effect and reinforcement from nicotine with each subsequent cigarette. Based on these findings, one would speculate that more dependent smokers will respond better (more compliance) to VLNC cigarettes.

Nicotine metabolism may also contribute to responses to RNC cigarettes. Nicotine is primarily metabolized by cytochrome P450 CYP2A (18, 19). Variations in enzyme activity and rate of nicotine metabolism appear to contribute to susceptibility to smoking (depending upon age), smoking rate, smoking intensity and quitting rate (e.g., 20, 21-25). For example, compared to fast nicotine metabolizers, slower metabolizers require fewer cigarettes to attain and maintain a desired level of nicotine (e.g., 20) and have been found to have better cessation rates when assigned to nicotine patches for cessation, presumably due to the attainment of higher nicotine levels (26). One can speculate that smokers with higher rates of nicotine metabolism (and higher rate of smoking) may have better response to VLNC cigarettes because of the stronger reinforcing value of sensory aspects of smoking as suggested by Brauer et al. (17).

Gender and race are also important factors to consider, particularly when considering responses to nicotine and success in quitting. Women have been observed to be less sensitive to nicotine, more sensitive to cues associated with smoking and to experience higher relapse rates in clinical trials (27-29). These studies would suggest that females may do better than males with VLNC cigarettes which provide the sensory aspects of smoking. Race also plays a role in smoking behavior and response. For example, compared to Caucasians, African Americans tend to smoke fewer cigarettes but cigarettes with higher tar and nicotine, make greater number of quit attempts but experience less success in cessation (30), and experience a higher incidence of smoking-related disease (31). In addition, African Americans have higher cotinine and cotinine concentrations per cigarette smoked (32-35), and slower metabolism of nicotine (36, 37). This finding, in part, may be due to the high rate of menthol cigarette use among African Americans and menthol has been

shown to inhibit glucuronidation and oxidation of nicotine and cotinine (38, 39). One study showed greater brain activation in response to smoking-related cues in African Americans compared to Caucasians (40). One could speculate that African Americans may have a better response (e.g., more compliance) to VLNC cigarette than Caucasians. In summary, there are a number of different factors that may contribute to the prediction of response to RNC cigarettes and these factors may also interact with each other. The possible predictors and interactions are likely to be many and would require a large sample size, however, an exploratory analyses is clearly indicated.

Need for proposed research:

The continued high prevalence of smoking and the high percent of smokers who have problems with quitting call for innovative approaches to tobacco control. Reducing nicotine content in cigarettes to the level that they are non-addictive may have a profound impact on public health. However, the best approaches for reducing levels of nicotine in cigarettes, effects of using these products on a long term basis and factors that may moderate responses to RNC cigarettes is virtually unknown. This study is the first to address these scientific gaps. The study will use the optimal threshold dose for “nicotine addiction” observed in Project 1 of the U54 DA031659, *Evaluating New Nicotine Standards for Cigarette* and will compare two nicotine reduction approaches on a comprehensive battery of measures. These measures include smoking behavior and subjective responses (including measures of abuse liability) and biomarkers of toxicant exposure and toxicity. No other studies have conducted such a thorough analysis comparing two different approaches to nicotine reduction.

OVERVIEW OF STUDY

The goal of this study will be to determine that method that potentially will result in the most public health benefit and the least harm to the smoker. This study will be conducted in 10 different academic institutions. Participants will be randomly assigned to: 1) immediate reduction to 0.03 mg nicotine yield or very low nicotine content (VLNC) cigarettes (optimal dose to be determined by Project 1 of the U54 grant; n=500 participants); 2) gradual reduction in nicotine content cigarettes, with each reduction occurring monthly (N=500 participants); and 3) experimental cigarettes with conventional levels of nicotine (0.8 mg nicotine yield; n=250 participants). Eligible participants will be blinded to the dose and provided the experimental cigarettes for a period of 5 months. Participants will complete questionnaires on demographics, smoking and health history, and drug and alcohol use history. Throughout the experimental phase, participants will record their use of tobacco products and complete questionnaires on mood and responses to the study tobacco product. Biomarker samples will be analyzed for exposure levels of nicotine and tobacco-related toxicants.

EXPERIMENTAL CIGARETTE DOSE

Cigarettes (Spectrum) will be provided by National Institute on Drug Abuse (NIDA) and dispensed by Research Triangle Institute (RTI). The following cigarette types are requested from NIDA:

Control condition: The 0.8 nicotine yield with 16.6 mg/g nicotine content and 10.5 mg tar yield for non-menthol and 0.8 nicotine yield with 16.08 mg/g nicotine content and 10.5 mg tar yield for menthol were chosen as the conventional cigarette yield because it is the dose most similar to full flavor cigarettes.

Immediate reduction condition: The nicotine yield of the cigarette will be 0.03 nicotine yield for menthol and non-menthol cigarettes (nicotine content is 1.3 mg/g; tar yield 8.0 and 8.2 mg, respectively)

Gradual reduction condition: The dosing for the gradual reduction will be the following:

Type	Nicotine Yield mg/cig	Tar Yield mg/cig
NRC600 CN	0.8 ± 0.15	9
NRC500 RN	0.7 ± 0.15	13.2
NRC400 RN	0.26 ± 0.06	9
NRC300 RN	0.12 ± 0.03	9
NRC102 RN	0.03 ± 0.01	9
NRC601 CN-Men	0.8 ± 0.15	9
NRC 501 RN-Men	0.6 ± 0.12	9
NRC401 RN-Men	0.26 ± 0.06	9
NRC301 RN-Men	0.12 ± 0.03	9

NRC103 RN-Men	0.03 ± 0.01	9
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CN= conventional nicotine; RN=reduced nicotine, Men=menthol

SCREENING PROCEDURES AND RISKS

Recruitment:

Cigarette smokers (n=1250) will be recruited from ten academic sites: University of Minnesota-Twin Cities, University of Minnesota-Duluth, Duke University, Johns Hopkins University, Oregon Research Institute, Mayo Clinic Scottsdale, MD Anderson Cancer Center, University of California-San Francisco, University of South Florida Moffitt Cancer Center and University of Pennsylvania. Participants will be recruited from advertisements through a variety of media outlets and the internet. As an example, the advertisement would read as follows: *Smokers who want to try new cigarettes that may or may not lead to reduced smoking are wanted for a research study. Participants will be paid for participation.* Cigarette smokers will contact the respective research centers and be screened for eligibility over the telephone. Participants must meet the following criteria for eligibility when screened over the telephone: Inclusion criteria: a) age > 18 years; b) smoking daily (average of ≥ 5 cigs per day for at least 1 year and no serious quit attempts (greater than 3 days) in the last 30 days; and c) no unstable medical or psychiatric conditions. Exclusion criteria: a) any changes in medications due to worsening of medical or psychiatric symptoms; b) regular use of tobacco other than cigarettes; c) current use of nicotine replacement or other tobacco products for cessation; and d) pregnant or breastfeeding. If participants meet the initial screening eligibility criteria for the study, they will be asked to attend an in-person screening session. Potential participants will be instructed to bring a pack of their usual brand cigarettes as well as all prescription medications they are currently taking to the screening visit.

Potential participants will be instructed to bring a valid government issued photo ID to the screening visit. Acceptable forms of identification include a Driver's License, a State Photo ID Card, Passport, or Military ID. If the potential participant does not have a valid photo ID, the interviewer can provide him/her with information on obtaining one.

A participant must complete his/her in-person screening session within 30 days of completing the telephone recruitment questionnaire. If the participant is not able to attend the in-person screening visit in that timeframe, he/she will need to complete the telephone recruitment questionnaire again.

Informed Consent Process:

Before beginning the informed consent process, valid state issued photo IDs will be examined and the age and identity of the potential participant will be confirmed. Persons who are not age 18 or older will be dismissed without payment. During the in-person screening session, study information will be presented and written informed consent will be required to participate in the screening. In order to ensure adequate informed consent, participants will be asked to read the first several lines aloud (to determine literacy) and will then be given ample time to read the consent document. If the interviewer suspects that the participant is not literate, he/she will read the informed consent to the participant. Inability to read and comprehend written study materials will result in ineligibility; however, if the participant consents, the interviewer will read the Tobacco Use History and Exposure and the Demographics questionnaires to allow comparisons of ineligible with eligible participants. The interviewer will use a standardized PowerPoint presentation to discuss the procedures, risks and benefits with the participant as well as their rights as a research participant. Participants will be told that they will be enrolled in a study where nicotine levels will change over time and the rate of change may vary across the three conditions. They will also be told that some of these study cigarettes may reduce their desire to smoke. Furthermore, we will describe the harmful effects of smoking and participants will be informed that they should feel free to quit smoking at any time during the intervention period. At the end of the presentation, the participants will be asked open-ended questions about the study and discuss the answers with the research staff person. Only after the participant and the research staff person are fully satisfied that the participant understands the purpose of the study, the confidentiality of the data, the procedures, the risks/benefits and their rights as a research participant will the consent form be signed and the participant undergo screening procedures.

Screening Measures:

Those who consent will be screened for eligibility using the following measures:

Physiological measures collected, recorded on paper, and entered into REDCap by the interviewer at the end of the visit:

- 1) Breath alcohol test to measure blood alcohol level (BAL) using an Alcosensor monitor. Participants with levels over 0.01 g/l may reschedule the interview but will need to be re-consented to ensure they have given adequate informed consent. They will be excluded if they are positive the second time.
- 2) Weight and height, will be measured to determine the participant's Body Mass Index. Changes in BMI will be assessed throughout the study. Weight will be measured in kilograms and height will be measured in centimeters.
- 3) Expired breath carbon monoxide (CO) levels will be assessed using a Micro+ Smokerlyzer® CO meter (Bedfont Instruments), a reliable and valid measure of recent smoking.
- 4) NicAlert Strips will be used to assess urinary cotinine levels if a participant's carbon monoxide reading is less than or equal to 8ppm.

- 5) A urine toxicological screen will be performed to assess the presence of illicit drugs including marijuana, cocaine, opiates, benzodiazepines, barbiturates, amphetamines, methadone, methamphetamines, and PCP. Participants who fail the drug screen (with the exception of marijuana) may reschedule the interview but will need to be re-consented to ensure they have given adequate informed consent. They will be excluded if they are positive the second time.
- 6) We will also ask the date of last menstrual period and length of cycle to assess pregnancy status (pregnancy tests [HCG detection] will be performed for female participants of childbearing potential prior to randomization at Baseline 2).
- 7) Blood pressure and heart rate will be measured using a CritiCare monitor to help the study physician determine final participant eligibility

The following screening questionnaires will be *participant-administered* via paper and then will be entered into the study databases by the interviewer after the visit:

- 1) Identifying Information Form will include the participant's REDCap Subject Identifier, name, address, email address, phone number, age and date of birth.
 - a. This form will be entered into an 'Identifying Information Database' by the interviewer.
 - i. Each site will have a separate 'Identifying Information Database'.
 - ii. Identifying information will not be shared with other sites. Each site is responsible for maintaining confidentiality of this information.
 - iii. Identifying information will be kept in a locked file cabinet (source document) and in a password protected Database (electronic version) separate from all other study data.
- 2) Brief Medical History Questionnaire to assess current diagnoses, symptoms and past health problems.
 - a. This questionnaire will be entered into REDCap by the interviewer.
 - b. The medications section will be transferred onto the 'Concomitant Medications' form and entered into REDCap by the interviewer.
- 3) Prime MD, (Patient Health Questionnaire) a brief questionnaire developed for evaluation of mental disorders by primary care physicians (41)
 - a. This questionnaire will be entered into REDCap by the interviewer.
- 4) Beck Depression Inventory 2nd Edition (BD-II; 42), if applicable, to assess depression in participants who endorse suicidal ideation or Major Depressive Disorder on the Prime MD
 - a. This questionnaire will be a **source document only**.

The following screening assessments will be *administered as an interview*:

- 1) Medical History Follow-Up Questionnaire, if applicable, to further assess current diagnoses, symptoms and past health problems
 - a. This questionnaire will be a **source document only**.
- 2) The Mini International Neuropsychiatric Interview (MINI) suicide subscale (43) to evaluate suicide risk
 - a. This questionnaire will be a **source document only**.
- 3) Tobacco Use History and Exposure Questionnaire, which measures variables such as smoking amount, cigarette brand, age of initiation of smoking, number of quit attempts, duration of quit attempts and duration of smoking
 - a. This questionnaire will be entered into REDCap by the interviewer after the visit.
- 4) Recreational Drug Use Questionnaire (12 + 1 month version)
 - a. This questionnaire will be entered into REDCap by the interviewer after the visit.
- 5) Smoking Cessation Therapy Use Questionnaire to assess current use or history of nicotine replacement therapy or smoking cessation medications to help participants quit smoking
 - a. This questionnaire will be entered into REDCap by the interviewer after the visit.

The following screening assessments will be *participant-administered via Qualtrics*:

- 1) Demographic History Questionnaire, which will assess age, gender, ethnicity, race, education, income, marital status, and employment history.
- 2) Alcohol Use Questionnaire (12 month version) (44)
- 3) Fagerström Test for Nicotine Dependence (45)
- 4) Smoking Stages of Change (46) as well as a contemplation ladder to assess intention to quit smoking
- 5) Center for Epidemiological Studies-Depression Scale (CES-D; 47), which measures symptoms of depression
- 6) Short Michigan Alcohol Screening Test (SMAST; 48), which measures past alcohol use
- 7) Drug Abuse Screening Test Brief Version (DAST-10; 49), which measures prior drug use and abuse

In the event that Qualtrics is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder and the interviewer will enter the data into Qualtrics when it resumes functioning properly.

Participants who make any response other than "not at all" on the suicidal ideation question of the Prime MD (Question 1i) or have a positive response to items 2-5 on the International Neuropsychiatric Interview (MINI) suicide subscale will not be eligible to participate in the study. To determine if a participant is in immediate danger, the research staff member will administer, on paper, the

Beck Depression Inventory 2nd Edition (BDI-II; 42) and refer to the licensed on-site clinician for evaluation. In the event that no clinician is available, staff will provide contact information for a 24-hour suicide prevention hotline (1-800-273-TALK). If in the clinician's or suicide counselor's opinion the participant presents an immediate danger to themselves or others, the investigator will call 911 and have an officer escort the participant to the nearest hospital. If in the clinician's or suicide counselor's opinion the participant does not present an immediate danger to himself/herself or others, the participant will be paid \$25, dismissed from the study, and provided a standard letter with resources and information about depression.

Additionally, any participant whose score on the Prime MD meets criteria for Major Depressive Disorder or a CES-D ≥ 16 will be administered the Beck Depression Inventory (BDI-II) on paper. The BDI-II will be submitted, along with the Prime MD, CES-D, Brief Medical History Questionnaire, Brief Medical History Follow-up Questionnaire and the MINI suicide subscale to the study clinician for eligibility review. If the study clinician determines a participant with Major Depressive Disorder or CES-D ≥ 16 to be eligible for study participation, the participant will complete the BDI-II on a weekly basis to monitor changes in his/her mood until deemed unnecessary by the appropriate health professional. A standard letter with mental health resources and information about depression will be provided to these individuals, if applicable.

Criteria for Study Eligibility:

Inclusion Criteria:

- 1) Age 18+
- 2) Daily smokers who smoke an average of at least five cigarettes per day for at least 1 year (no more than 30 days continuous abstinence)
- 3) Breath CO levels > 8 ppm (if ≤ 8 ppm, then NicAlert Strip level must indicate regular smoking)

Exclusion Criteria:

- 1) Planned quit date in the next 30 days
- 2) Currently seeking treatment for smoking cessation
- 3) Currently using nicotine replacement therapies or other pharmacotherapies as cessation aid (non-cessation intermittent use acceptable)
- 4) A quit attempt in the past 30 days resulting in greater than 3 days of abstinence
- 5) Using other tobacco products or e-cigarettes more than 9 days in the past 30 days
- 6) Significant unstable medical conditions (Any significant change in a serious medical condition occurring during the past 3 months including, cardiovascular disease, COPD, and cancer, as determined by the licensed medical professional at each site)
- 7) Unstable psychiatric conditions (Any significant change in psychiatric symptoms during the past 3 months as determined by the licensed medical professional at each site)
- 8) Schizophrenia and schizoaffective disorder
- 9) Psychiatric medication changes (e.g., new prescriptions, changes in dosages, or discontinuation of medications) in the past 3 months that was a result of negative changes in symptoms.
- 10) Positive toxicology screen for any of the following drugs: cocaine, opiates, methadone, benzodiazepines, barbiturates, amphetamines, methamphetamines, and PCP
- 11) Marijuana will be tested for but will not be an exclusionary criterion.
- 12) Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines or methadone will not be excluded.
- 13) Participants failing the toxicology screen will be allowed to re-screen once.
- 14) Blood alcohol level > 0.01
- 15) Participants failing the blood alcohol screen will be allowed to re-screen once.
- 16) Binge drinking alcohol (more than 9 days in the past 30 days, 4/5 drinks per day (female/male))
- 17) Pregnant, trying to become pregnant or breastfeeding
- 18) Predominant use of 'roll your own cigarettes'
- 19) CO reading > 80 ppm
- 20) Systolic BP greater than or equal to 160
- 21) Participants failing for blood pressure will be allowed to re-screen once.
- 22) Diastolic BP greater than or equal to 100
- 23) Participants failing for blood pressure will be allowed to re-screen once.
- 24) Systolic BP below 90 and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
- 25) Participants failing for blood pressure will be allowed to re-screen once.
- 26) Diastolic BP below 50 and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
- 27) Participants failing for blood pressure will be allowed to re-screen once.
- 28) Heart rate greater than or equal to 105 bpm
- 29) Participants failing for heart rate will be allowed to re-screen once.
- 30) Heart rate lower than 45 bpm and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)

- 31) Participants failing for heart rate will be allowed to re-screen once.
- 32) Indicating any suicidal ideation in the past month, suicide attempts in the past 5 years (if within past 5 to 10 years, requires licensed medical professional approval), or score of >4 on the MINI suicide subscale
- 33) Household member enrolled in the study concurrently.
- 34) Inability to independently read and comprehend the consent form and other written study materials and measures because participants are required to complete parts of the protocol at home independently.
- 35) Participated in prior study that involved reduced nicotine content cigarettes within the past 3 years.
- 36) Having participated in a research study during the past three months in a study that would impact baseline smoking or response to study products.
- 37) Currently taking the following anticonvulsant medications:
 - a. Phenytoin [Brand Name: Dilantin]
 - b. Carbamazepine [Brand Name: Tegretol, Carbatrol, Equetro, Epitol]
 - c. Oxcarbazepine [Brand Name: Trileptal]
 - d. Primidone [Brand Name: Mysoline]
 - e. Phenobarbital
- 38) Currently taking the following medication:
 - a. Bendamustine (Treanda)
 - b. Clopidogrel (Plavix)
 - c. Clozapine (Clozaril, FazaClo)
 - d. Erlotinib (Tarceva)
 - e. Flecainide (Tambocor)
 - f. Fluvoxamine (Luvox)
 - g. Irinotecan (Camptosar)
 - h. Olanzapine (Zyprexa)
 - i. Ropinirole (Requip)
 - j. Tacrine (Cognex)
 - k. Theophylline (Theo Dur, etc.)
- 39) Unstable living condition that would compromise compliance to study procedure.

Justification for Inclusion and Exclusion Criteria:

Children under age 18 are excluded because they cannot legally buy cigarettes. Those with unstable medical or medication conditions (significant change in condition and/or medication changes in the past 3 months as determined by medical professional) are excluded as these symptoms could affect a participant's ability to complete the study. Examples include but are not limited to the following: angina, stroke, heart attack which occurred since phone screening, blood clots in the arms or legs for which the individual is undergoing active medical treatment, cancer requiring active chemotherapy or radiation therapy, fractured limbs, severe arthritis, or other debilitating conditions of mobility, severe shortness of breath caused by conditions such as uncontrolled asthma, COPD, or arrhythmia, active untreated infection such as pneumonia, active untreated endocrine disorder such as hyperthyroidism, or any other condition which is likely to impair the individual's ability to attend study visits. We will exclude those currently seeking smoking treatment, those who have quit smoking for longer than 3 days in the past 30 days or are planning to quit in the next 30 days, as participation in this study may not lead to reductions in smoking. Pregnant and nursing women and anyone with current or recent alcohol or drug abuse problems will be excluded as these factors could independently affect smoking behavior during the study. Individuals with baseline CO readings greater than 80 ppm, those with heart rate or blood pressure readings that are out of range and anyone who has attempted suicide in the past 5 years will be excluded from the study for safety concerns. Individuals who only smoke 'roll your own' cigarettes will be excluded from the study because we will be unable to standardize their baseline smoking behavior. If an individual has recently participated in a smoking research study that changed their smoking behavior this person would be excluded because they would not have a stable smoking baseline. Additionally, participants who have had experience with RNC cigarettes within the past 3 years will be excluded from the study. Use of certain medications that may interact with tobacco smoke or nicotine will be excluded since nicotine reduction or smoking cessation may impact metabolism of these medications.

Eligibility Determination:

The final eligibility of the participant will be determined by a licensed medical professional (MD, NP, etc.) at each site after reviewing the Brief Medical History Questionnaire, Medical History Follow-Up Questionnaire and the MINI suicide subscale. If the participant's score on the Prime MD indicates a psychiatric disorder then the Prime MD will be submitted to the medical professional for review as well. Additionally, if the participant's score on the Prime MD indicates Major Depressive Disorder, the Prime MD along with the Beck Depression Inventory 2nd Edition will be submitted to the medical professional for review. The licensed medical professional may meet with a participant if available and think it necessary for eligibility determination. He/she will sign off on eligibility prior to the first baseline visit. If the medical professional determines the participant is not medically eligible to participate

in the study, he/she will inform the research assistants who will contact the participant. The study medical professional will NOT need to review the medical history forms of participants who are not eligible for other, non-medical reasons.

During the telephone screening, all eligible participants will be instructed to bring any prescription medications they are currently taking to their in-person screening visit. If a participant fails the urine toxicology screen due to a prescription medication they are taking (including methadone maintenance), they will not be automatically excluded. The interviewer will record the positive result into REDCap and perform an eligibility override to prevent the system from marking the participant as ineligible. The interviewer will make note of this when they submit the forms to the study medical professional for final eligibility determination.

At the end of the screening session, the investigator will complete an End of Visit Evaluation, which will be entered into REDCap. This evaluation will allow the investigator to make note of any problems encountered during the visit and to assess the truthfulness of the participant in regards to self-report of tobacco use.

Potential Risks of Participation:

- 1) *Survey Questionnaires:* The interview will include questions about medical history, drug and alcohol use, breath sample measures of alcohol and cigarette use, urine tests of drug use and pregnancy and questionnaires about mood. Answering these personal questions could make the participant feel uncomfortable.
- 2) *Breach of Confidentiality:* The risk of the interview is loss of privacy if other people find out the results.
- 3) *Drug Testing:* A breach of confidentiality could occur and other people could learn of the participant's drug use.
- 4) *Obtaining blood pressure:* The blood pressure cuff may cause minimal discomfort. In obtaining blood pressure, researchers may find out the participant has abnormal blood pressure.
- 5) *Smoking cigarettes:* All cigarettes are detrimental to a person's health and can lead to the following medical problems:
 - a. Cardiovascular Disease: Coronary heart disease, heart attack, stroke, peripheral vascular disease, reduced blood circulation, abdominal aortic aneurysm
 - b. Respiratory Diseases: Emphysema, bronchitis, tuberculosis, and chronic airway obstruction
 - c. Cancers: Lung, bladder, liver, colon, cervical, esophageal, kidney, larynx, mouth, pancreatic, throat, stomach cancers and acute myeloid leukemia
 - d. Diabetes
 - e. Immune function, rheumatoid arthritis
 - f. Other Health Risks: Infertility, ectopic pregnancy, preterm delivery, stillbirth, low birth weight, congenital defects (orofacial cleft) sudden infant death syndrome (SIDS), lower bone density in postmenopausal women, and increased risk for hip fracture in women; male sexual dysfunction; age-related macular degeneration, blindness, cataracts
- 6) *Smoking study cigarettes:* In addition to the above medical problems, participants may experience some minor adverse health effects such as headaches or experience withdrawal symptoms which are listed below. In addition, due to the altered nicotine levels, there could be a change in their cigarette use including the manner in which they inhale the smoke or increase the number of cigarettes smoked per day. This increased rate of smoking may persist after completing the study. Subjects will be assessed for increased rate of smoking at follow-up, advised to quit smoking and provided cessation referrals. Smoking the study cigarettes does not necessarily provide any less risk than their usual brand cigarette and could pose increased health risks. Participants may also experience increases in carbon monoxide, a gas from smoke. The study cigarettes are made from genetically modified tobacco plants. A full toxicological evaluation has not been conducted, thus the consequences of inhaling this genetically modified product is unknown.
- 7) *Smoking Withdrawal:* Participants may experience smoking withdrawal symptoms during this study. The symptoms can be uncomfortable but are of minimal risk. At each visit, we will ask how the participant feels, and if we think that being in this study is putting his/her health at risk, we may ask the participant to stop participating in the study. Smoking withdrawal symptoms include:
 - a. Anger, irritability, frustration
 - b. Anxiousness, nervousness
 - c. Depressed mood or sadness
 - d. Desire or craving to smoke
 - e. Difficulty concentrating
 - f. Increased appetite, hunger or weight gain
 - g. Insomnia, problems sleeping or awakening at night
 - h. Restlessness
 - i. Impatience
 - j. Constipation
 - k. Dizziness
 - l. Coughing
 - m. Dreaming or nightmares
 - n. Nausea
 - o. Sore Throat

- 8) *Returning to regular smoking*: It is possible that if participants return to smoking their usual brand of cigarettes at the end of the study they may experience mild and transient nausea, dizziness, and lightheadedness.
- 9) *Risk to Fetus*: To avoid risks to a fetus, pregnant women will be excluded from this study. Risks include miscarriage, preterm delivery, stillbirth, low birth weight, problems with the placenta, birth defects such as cleft palate, sudden infant death syndrome (SIDS) and early childhood behavioral problems.
- 10) *Changes in blood pressure and/or heart rate*: Smoking and nicotine can affect the cardiovascular system which may result in changes in blood pressure and/or heart rate.
- 11) *Exacerbation of psychiatric symptoms*: Smoking and nicotine can affect a person's mood and emotions and are associated with psychiatric disorders including major depressive disorder, general anxiety disorder, bipolar disorder and eating disorders. Any changes in nicotine or cigarette consumption could adversely affect psychiatric conditions.
- 12) *Risks associated with bio-sample collection*: Venipuncture may result in bruising, infection, fainting or dizziness).
- 13) *Changes in therapeutic levels for certain medications due to drug and smoking interaction*: Certain medications may be impacted by cigarette smoking and dosage may need to be monitored by the participant's health professional. A list of common medications potentially affected will be provided to the site's licensed medical professional.

Avoiding Risks to Fetus:

If participants choose to be sexually active, they should use an appropriate "double barrier" method of birth control (such as female use of a diaphragm, intrauterine device (IUD), or contraceptive sponge, in addition to male use of a condom) or the female should be using prescribed "birth control" pills, injections or implants. Female participants with child-bearing potential will be tested for pregnancy at the randomization visit, at Week 4, 8, 12, 16 of the experimental period and at Week 20 at the end of the experimental period. If a participant becomes pregnant during the study she will be withdrawn from the study. Approximately 30 days after being withdrawn, the research staff will contact the participant to confirm her due date. The licensed medical professional will follow-up with the participant after delivery to ask questions about the baby's health.

Expected Benefits of Participation:

There are no immediate benefits from participating in the study. The information obtained from this study may ultimately help the Food and Drug Administration decide how best to regulate tobacco products with the goal of improving public health.

STUDY PROCEDURES

Overview:

Participants will undergo 2 weeks of baseline smoking and then be randomized to one of the three experimental conditions: 1) Control; smoking 0.8 mg nicotine yield cigarette; 2) Immediate reduction to 0.03 mg nicotine yield cigarette; and 3) gradual reduction to 0.03 mg nicotine yield cigarettes. The experimental period will be 20 weeks. During this period, participants must be willing to attend weekly clinic visits for 4 weeks and bi-weekly between weeks 5-20. Visits should occur at consistent times during the day (e.g., morning, midday or evening). Participants and staff will be blinded to the condition to which they are assigned. At the end of 20 weeks, participants will be encouraged to quit smoking and provided cessation materials and referrals.

Baseline Period:

This study will use a two-week, two session baseline period to collect baseline individual difference measures and monitor daily usual-brand smoking behavior. During the baseline period, participants will NOT be provided their usual brand cigarettes to smoke. Use of a two session baseline period will ensure stability of daily smoking reports and reduce reactivity to the daily cigarette monitoring. During the two baseline sessions, participants will complete subjective questionnaires, be assessed on physical measures and submit biosamples. Each visit will last approximately two hours. At the end of each baseline session, the investigator will complete an End of Visit Evaluation, which will be entered into REDCap. This evaluation will allow the investigator to make note of any problems encountered during the visit and to assess the truthfulness of the participant in regards to self-report of tobacco use.

Visit Scheduling Requirements

Participants will be required to schedule the Baseline 1 visit within 30 days of their screening visit. If a participant still wants to be in the study after 30 days, he or she will need to be re-screened. The participant will need to be re-consented but will maintain the original assigned Subject Identifier. The ideal target window separating Baseline 1 and Baseline 2 is between 6-12 days. The minimum is 6 days and the maximum is 21 days. If the participant does not complete the visit within 21 days, then either he/she will be rescheduled if this delay was a result of a legitimate excuse (e.g., family emergency) or will be discontinued from the study.

Baseline Measures (Please see Table 1 for measurements and schedule of administration)

Baseline 1 & 2 physiological measures, recorded on paper, and entered into REDCap by the interviewer after the visit:

- 1) BAL
- 2) Weight
- 3) CO
- 4) Blood Pressure

5) Heart Rate

The following assessments will be *administered as an interview* and then entered into the study data base by the interviewer after the visit:

Baseline 1 & 2

- 1) Concomitant Medications Questionnaire – Will be entered into REDCap by the interviewer after the visit.
- 2) Adverse Event Form, if applicable, will assess the nature, severity, duration, action taken, and outcome of events related to tobacco product use (participants will also be given contact cards so that they can inform us of events that occur between study contacts) – Will be entered into REDCap by the interviewer after the visit.
- 3) Health Changes Questionnaire, which will assess any weekly health changes – Will be entered into REDCap by the interviewer after the visit.
- 4) Timeline Follow Back Questionnaire,(50) which will assess other tobacco and nicotine product use as well as alcohol and marijuana use since the last study visit– Will be entered into REDCap by the interviewer after the visit.

Baseline 2 Only

- 1) Pregnancy Tests (HCG detection) will be performed for female participants of childbearing potential. We will also confirm the date of last menstrual period and length of cycle.
- 2) Drug Use Questionnaire, 1 Month version - Will be entered into REDCap by the interviewer at the end of the visit.

Assessments *administered as an interview and kept as source documents* only

- 1) BDI-II, if applicable

The following assessments will be completed by the *participant using Qualtrics* in order to standardize assessments across sites and projects:

Baseline 1 Only

- 1) Wisconsin Index of Smoking Dependence Motives-Brief (WISDM; 51), a measure of tobacco dependence
- 2) Perceived Health Risks Rating - Baseline (16), a measure of the perceived addictive potential and other health risks associated with cigarettes
- 3) Positive and Negative Affect Schedule (PANAS; 52).

Baseline 1 & 2 assessments administered using Qualtrics

- 1) Respiratory Health Questionnaire, a measure of cough, shortness of breath and other respiratory symptoms
- 2) Minnesota Nicotine Withdrawal Scale (MNWS; 53), a measure of nicotine withdrawal
- 3) Questionnaire of Smoking Urges-brief scale- Usual Cigarette (QSU; 54, 55), which measures the urge to smoke
- 4) Cigarette Evaluation Scale - Baseline (CES; 56), which measures responses to cigarettes (e.g., reward, satisfaction)

Baseline 2 Only assessments administered using Qualtrics

- 1) Drug Effects/Liking Scale (57)
- 2) Environmental and Social Influences on Tobacco Use Questionnaire (58), which measures tobacco smoke exposure at home, work and socially
- 3) Cigarette Purchase Task – Usual Brand Version (59) which will be used to generate cigarette demand curves. Participants will be asked to report the number of cigarettes that they would consume in a day at various costs. Several indices of demand are generated from the raw values, including demand intensity (consumption at zero price), Omax (maximum amount of money allocated to cigarettes), breakpoint (the first price at which a participant reports zero consumption) and Pmax (the price at which Omax occurs). This task will indicate whether prolonged VLNC use reduces cigarette demand and increases sensitivity to increases in cigarette costs. During the Baseline 2 visit participants will complete the Cigarette Purchase Task- Usual Brand Cigarette Version only.
- 4) Alcohol Use Questionnaire, 1 month version
- 5) Perceived Stress Scale (60), which measures the degree to which life situations are appraised as stressful

In the event that Qualtrics is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder and the interviewer will enter the data into Qualtrics when it resumes functioning properly.

Baseline 2 biological specimens collected, stored, and tracked in the Biosample Database:

- 1) Urine sample for smoking biomarker assessment:

Participants will be asked to bring a urine sample (first void of the day) to the second baseline session for biomarker assessment. Samples will be analyzed for the following biomarkers and remaining samples will be sent to a Biorepository with de-identified information. The carcinogen biomarkers of exposure and effect were chosen because they have shown reasonable laboratory reproducibility, have clear differences in levels between smokers and nonsmokers and/or decrease upon tobacco cessation (61, 62). The tobacco-specific carcinogen biomarkers are total NNAL and total NNN. Total nicotine equivalents (TNE) was chosen to assess

nicotine exposure. TNE accounts for 73-96% of the nicotine dose and is a useful measure of daily nicotine exposure. We may also assess minor alkaloids, including anatabine, anabasine, myosmine, and normicotine, which may contribute to the reinforcing effects of cigarettes. In addition we may assess 8-epi PGF_{2α} as well as phenanthrene tetroal and mercapturic acids of acrolein, benzene, 3-butadiene, crotonaldehyde, and ethylene oxide. Samples will be stored at temperatures no more than -20°C. Urine samples will be sent as requested to be analyzed and stored at the University of Minnesota. If a participant forgets to bring his/her urine sample to the Baseline 2 visit, the visit will be postponed until the participant brings in their first void samples.

2) Blood sample for cardiovascular risk assessment and nicotine metabolite ratio (NMR):

Participants will provide a blood sample that will be analyzed for C-reactive protein and white blood count (WBC) (15, 16). Serum will be used to assess NMR, an indicator of CYP2A6 enzyme activity (18-20) and a potential predictor of responses to RNC and VLNC cigarettes. NMR is the ratio between two nicotine metabolites, cotinine and trans-3'-hydroxycotinine (3-HC). Bloods for C-reactive protein will be processed, frozen and shipped in batches at a later date to University of Minnesota. The hemogram will be analyzed at a local lab.

3) Oral cell sample collected from the mouth:

Oral cells will only be collected from subjects at the University of Minnesota Twin Cities and Duluth sites. Oral cells can serve as a minimally invasive tool to measure the responses to alternative tobacco products and can be leveraged to understand the underlying biological mechanisms that are similar and different from smoking cessation (63). Cells from the mouth will be gently scraped from the inside of the cheek with a cytobrush. Samples will be stored at -20°C or less and will be shipped to University of Minnesota.

4) Saliva sample for DNA analysis:

Participants will be asked to provide a baseline saliva sample for possible future DNA analysis. The sample will be shipped and stored at the University of Minnesota.

Baseline 2 cigarette butts collected, stored, and shipped to the CDC:

1) 24-hour cigarette butt collection:

Cigarette butts will be collected for a subset of subjects at a subset of sites (Moffitt Cancer Center, UCSF and UMN Twin Cities) during the 24 hours prior to the biosamples first void urine collection on a subset of participants. Participants will be provided with vials and a collection box and will be instructed to put the cigarette butts into the collection box in the order they were smoked. Both study and non-study cigarettes will be collected. If the participant forgets to collect a cigarette butt he/she will be instructed to skip a space in the collection box to indicate a missing sample. Samples will be stored at room temperature. Cigarette butts will be sent to the CDC for solanesol analysis, another indicator of puffing behavior and possible compensatory smoking.

Interactive Voice Response System:

At the Screening visit, participants will be trained to use the Interactive Voice Response (IVR) System, which will contact participants each day throughout the study and ask about their smoking behavior (usual brand during the two week baseline and study and non-study cigarettes during experimental period) and a shortened version of withdrawal symptoms. Participants will be instructed that if the relight a cigarette, that cigarette would still be considered as one cigarette. Participants will be provided a study cell phone if they have unreliable telephone access, do not have enough monthly cell phone minutes or just prefer not to use their own phone.

To be enrolled in the IVR system, research staff will enter the participant's initials, telephone number, subject identifier and visit dates into the HIPAA compliant IVR website. Identifying information (initials and telephone numbers) will not be extracted with the data by the bioinformatics group.

Experimental Period:

Randomization:

After 2 weeks' baseline smoking, participants will be randomized to one of three experimental conditions based on a pre-determined random number sequence generated by Dr. Xianghua Luo and stored in the study central database program. Randomization will be stratified by site. This ensures a similar distribution of treatment assignment (2:2:1 for immediate reduction: gradual reduction: control) across study sites to avoid potential confounding.

Overview of Visits:

Subjects visits will be once a week for the first 4 weeks (Weeks 1 through 4) and then every other week for the next 16 weeks (8 visits: Week 6, Week 8, Week 10, Week 12, Week 14, Week 16, Week 18 and Week 20). We will attempt to schedule visits at consistent times of the day. At the end of 20 weeks, participants will be encouraged to quit smoking and offered treatment materials. At the end of each session, the investigator will complete an End of Visit Evaluation, which will be entered into REDCap. This evaluation will allow the investigator to make note of any problems encountered during the visit and to assess the truthfulness of the participant in regards to self-report of tobacco use.

Visit Scheduling Requirements for Experimental Period:

The scheduling window for visits 1 through 4 is 7 days \pm 3 days from target date. The window for visits 6 through 20 is 14 days \pm 5 days from target date, and the window for the follow-up visit is 28 days -5 or +7 days from target date. If the participant's visit falls outside this range it is a protocol deviation and will need to have a 'Protocol Deviation Form' completed, tracked in REDCap and submitted to the Coordinating Center (University of Minnesota).

If a participant is not able to attend the final experimental visit (Week 20) within the designated scheduling window, it should nonetheless be rescheduled and documented as a protocol deviation.

Product Dispensing:

During the experimental period, participants will be provided with a 14-day supply of research cigarettes during weekly visits and 21-28-day supply during bi-weekly visits. This will ensure adequate availability of cigarettes in the numerous locations participants may typically keep a supply (home, work, vehicle, etc.) as well as avoid expending the entire supply if they miss a scheduled visit. If there is prior knowledge a participant will be missing a visit (i.e. planned vacation, clinic/laboratory closure, etc.), then the participant will be provided with an adequate supply of cigarettes to make up for the missed visit(s).

Participants will be asked to refrain from use of other non-study tobacco products during the study period. They will be told that it is crucial for them to abstain from any use of non-study cigarettes or other nicotine or tobacco products. To increase the incentive to exclusively use the study cigarettes, a semi-bogus pipeline will be used. Subjects will be told that some of their spot urine samples will be tested to verify no other nicotine exposure has occurred. Brief standardized review session that focuses on compliance with the experimental cigarettes and other study procedures will be provided at each visit.

Product Accountability:

Participants will be required to keep track of all the cigarettes provided to them. Therefore, they will be instructed to return all unused cigarettes and empty cigarette packs to the clinic/laboratory each week. Research staff will fill in the 'Product Accountability Log' with the participants. Any discrepancies in the product dispensed versus product returned will be discussed and recorded in the log. Empty cigarette packs will not be saved; however, research staff will keep all empty cartons in storage for reference. All unused study cigarettes will be collected from participants at all visits. Unused cigarettes will be re-distributed to the participants only during the weeks of the experimental period where there is no change in nicotine yield (week 1, 2, 3, 6, 10, 14, and 18). During Week 20, any remaining unused cigarettes returned by the participants will be collected by the research staff.

During the experimental period, participants will be provided a nominal amount of money for each unused pack of cigarettes that are returned in order to disincentivize wasted use of cigarettes. Subjects who return $\geq 25\%$ of their cigarette packs unopened, will receive a \$5 credit each visit that will be paid out after the follow-up visit.

Measures during Experimental Period (See Table 1 for measurements and schedule of administration)

Experimental Procedures for Visits at Weeks 1, 2, 3, 6, 10, 14 and 18:

Physiological Measures Collected, recorded on paper, and entered into REDCap by interviewer after the visit:

- 1) BAL
- 2) Weight
- 3) CO
- 4) Blood Pressure
- 5) Heart Rate
- 6) Spot urine sample for possible random compliance check (not recorded in REDCap)

The following assessments will be *administered as an interview* and will be entered into the study databases by the interviewer after the visit:

- 1) Concomitant Medications - Will be entered into REDCap by the interviewer.
- 2) Adverse Event Form, if applicable - Will be entered into REDCap by the interviewer.
- 3) Health Changes Questionnaire - Will be entered into REDCap by the interviewer.
- 4) Timeline Follow Back Questionnaire (for assessing marijuana, alcohol and tobacco/nicotine products) - Will be entered into REDCap by the interviewer.

Assessments *administered as an interview* and kept as source documents only

- 1) BDI-II, if applicable

The following assessments will be completed by the *participant using Qualtrics*:

- 1) Respiratory Health Questionnaire
- 2) Minnesota Nicotine Withdrawal Scale

- 3) Questionnaire of Smoking Urges brief scale - Usual Cigarette
- 4) Questionnaire of Smoking Urges brief scale - Experimental Cigarette
- 5) Cigarette Evaluation Scale - Experimental (Weeks 1, 2, 3 only and not 6, 10, 14, 18)
- 6) Drug Effects/Liking Scale (Weeks 1, 2, 3 only and not 6, 10, 14, 18)

Experimental Procedures for Visits at weeks 4, 8, 12, 16 and 20:

Physiological measures collected, recorded on paper, and entered into REDCap by interviewer after the visit:

- 1) BAL
- 2) Weight
- 3) CO
- 4) Blood Pressure
- 5) Heart Rate
- 6) Urine sample for biomarkers (first morning void)
- 7) Spot urine sample for drug test (Week 20 only), pregnancy test
- 8) Spot urine for possible random compliance check (not in REDCap)
- 9) Blood samples (not in REDCap)
- 10) Oral cells (mouth) if applicable (not in REDCap)

The following assessments will be *administered as an interview* and will be entered into the study databases by the interviewer after the visit:

- 1) Concomitant Medications - Will be entered into REDCap by the interviewer.
- 2) Adverse Events Form, if applicable - Will be entered into REDCap by the interviewer.
- 3) Drug Use Questionnaire, 1 Month version - Will be entered into REDCap by the interviewer.
- 4) Health Changes Questionnaire - Will be entered into REDCap by the interviewer.
- 5) Timeline Follow Back Questionnaire (for assessing marijuana, alcohol and tobacco/nicotine products) - Will be entered into REDCap by the interviewer.

Assessments *administered as an interview and kept as source documents only*

- 1) BDI-II, if applicable

The following assessments will be completed by the *participant using Qualtrics*:

- 1) Respiratory Health Questionnaire
- 2) Minnesota Nicotine Withdrawal Scale
- 3) Questionnaire of Smoking Urges-brief scale - Usual Cigarette
- 4) Questionnaire of Smoking Urges-brief scale - Experimental Cigarette
- 5) Cigarette Evaluation Scale - Experimental
- 6) Drug Effects/Liking Scale
- 7) Perceived Health Risks Questionnaire – Experimental
- 8) Perceived Stress Questionnaire
- 9) Positive and Negative Affect Schedule (PANAS)
- 10) Cigarette Purchase Task – Usual Brand Cigarette
- 11) Cigarette Purchase Task – Experimental Brand Cigarette
- 12) CES-D
- 13) PrimeMD (Patient Health Questionnaire) will be done only at week 20
- 14) FTND
- 15) NIAAA Alcohol Use Questionnaire, 1 month version
- 16) Wisconsin Index of Smoking Dependence Motives-Brief
- 17) Environmental and Social Influences on Tobacco Use Questionnaire (week 20 only)
- 18) Smoking Stages of Change Algorithm and Contemplation Ladder (week 20 only)
- 19) Short Michigan Alcohol Screening Test (SMAST) (week 20 only)
- 20) Drug Abuse Screening Tool Brief Version (DAST) (week 20 only)
- 21) End of Study Questionnaire & Evaluation (week 20 only)

In the event that Qualtrics is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder and the interviewer will enter the data into Qualtrics when it resumes functioning properly.

Biological Specimens to be collected, stored, and tracked in the Biosample Database:

- 1) *Urine sample* for smoking biomarker assessment:

Participants will be asked to bring a urine sample (first void of the day) to the weeks 4, 8, 12, 16 and 20 visits for biomarker assessment. Samples will be stored at temperatures no more than -20°C. Urine samples will be sent as requested to be analyzed and

stored at the University of Minnesota. If a participant forgets to bring his/her urine sample, then a spot urine sample will be collected and noted.

2) *Blood sample* for cardiovascular risk factor assessment:

Blood will be drawn by a phlebotomist or trained personnel and sent for hemogram analysis at a local laboratory. Bloods for C-reactive protein will be processed, frozen and shipped in batches at a later date to University of Minnesota.

3) *Oral cell sample* collected from the mouth (if applicable):

Oral mucosa scrapings will be obtained by trained personnel. Cells from the mouth will be gently scraped from the inside of the cheek with a cytobrush. Samples will be stored at -20°C or less and shipped to University of Minnesota

Specimens to be collected, stored and shipped to the CDC

1) 24-hour cigarette butt collection: Cigarette butts will be collected for a subset of subjects at a subset of sites (Moffitt Cancer Center, UCSF and UMN Twin Cities) during the 24 hours prior to the biosamples first void urine collection on a subset of participants. Participants will be provided with vials and a collection box and will be instructed to put the cigarette butts into the collection box in the order they were smoked. Both study and non-study cigarettes will be collected. If the participant forgets to collect a cigarette butt he/she will be instructed to skip a space in the collection box to indicate a missing sample. Samples will be stored at room temperature. Cigarette butts will be sent to the CDC for solanesol analysis, another indicator of puffing behavior and possible compensatory smoking.

Interactive Voice Response System:

Participants will continue to use the IVR system on a daily basis throughout the treatment period to record the number of research cigarettes smoked per day and use of non-research cigarettes. During the baseline period and through week 21 IVR will collect information from the participants about withdrawal symptoms.

Product and Procedures Compliance Review Sessions:

At each visit, participants will be counseled about their use of the assigned cigarettes. Participants will be asked about any concerns or obstacles associated with use of the assigned cigarettes. The importance of honest reporting will be stressed. Participants will be told not to use other nicotine or tobacco products but that it is crucial for them to report any use of these products. If difficulties are encountered, participants will be asked why they think they are experiencing difficulties (e.g., taste, withdrawal symptoms) and to problem-solve how to deal with these difficulties in order to meet the protocol requirements. To encourage abstinence from other tobacco or nicotine products, subjects will be informed that we will randomly check the spot urine sample collected at each visit. Additionally, participants will be counseled about their IVR completion, visit attendance and product accountability. Each review session should have a goal of 10 minutes. The minimum requirement is five minutes.

Quit Attempts During the Study Protocol:

At each weekly session, we will ask the participant if he/she is currently abstaining from smoking and/or has the intention of quitting or planning to quit smoking prior to his/her next scheduled visit. If the answer is yes, they will be provided with information on quitlines to assist them in their quit attempt.

If a Participant is Currently Abstaining from Smoking with the Intention to Quit:

- Encourage participant to continue abstaining from smoking
- Schedule the participant for normal weekly or bi-weekly visits
- Provide the participant with the 'Clearing the Air' manual and local smoking cessation resources
- Give the participant the option to take home study product rather than require him/her to take the product
- If the participant chooses to take home the study product have him/her sign a form acknowledging that cigarette availability could be detrimental to the quit attempt
- Recommend that he/she puts the product "away" at home as to avoid unwanted cues to smoke
- If the participant chooses not to take home the study product, have him/her contact the clinic/lab if he/she lapses and would like to pick up or be mailed the study product prior to his/her next visit

If a Participant is Planning to Quit Smoking, But Has Not Initiated the Quit Attempt:

- Ask if he/she has identified a target quit date and, if so, what the target date is
- Provide the participant with the 'Clearing the Air' manual and local smoking cessation resources Provide the participant with the study product as usual. Recommend that on the target date he/she puts the product "away" at home so as to avoid unwanted cues to smoke

Follow-Up Session:

After the week 20 visit, participants will be required to come back for one additional visit 4 weeks (28 days -5, +7 days) after completion. Tobacco use status (amount and type of tobacco/nicotine product use) will be determined and urine samples assessed for total nicotine equivalents (TNE) levels.

Measures

Physiological Measure Collected, recorded on paper, and entered into REDCap by interviewer after the visit:

- 1) BAL
- 2) Weight
- 3) CO
- 4) Blood Pressure
- 5) Heart Rate

The following assessments will be *administered as an interview* and will be entered into study databases by the interviewer after the visit:

- 1) Concomitant Medications – Will be entered into REDCap by the interviewer.
- 2) Adverse Event Form, if applicable - Will be entered into REDCap by the interviewer.
- 3) Health Changes Questionnaire - Will be entered into REDCap by the interviewer.
- 4) Timeline Follow Back Questionnaire - Will be entered into REDCap by the interviewer.
- 5) Follow-up Questionnaire – Will be entered into REDCap by the interviewer.

The following assessments will be completed by the *participant using Qualtrics*:

- 1) Fagerström Test for Nicotine Dependence
- 2) Smoking Stages of Change as well as a contemplation ladder to assess intention to quit smoking
- 3) Cigarette Evaluation Scale – Usual brand

Biological specimens to be collected, stored, and tracked in the Biosample Database:

- 1) Urine sample for smoking biomarker assessment:

Participants will be asked to bring a urine sample (first void of the day) to the follow-up visit for biomarker assessment. Samples will be stored at temperatures no more than -20°C. Urine samples will be sent as requested to be analyzed and stored at the University of Minnesota. If a participant forgets to bring his/her urine sample, then a spot urine sample will be collected and noted.

If urinary biomarker levels from the Week 24 visit are substantially elevated over baseline levels and it is determined this may be a safety issue, participants may be asked to come to the clinic after returning to their usual brand for a sustained period of time. At this visit, they will provide an additional urine sample and tobacco use and health status. If levels continue to be elevated, participants will be strongly encouraged to quit smoking provided tobacco cessation resources.

Early Termination:

If a participant decides to withdraw from the study prior to completion or if they are withdrawn by the PL due to safety concerns, they will be scheduled for an early termination visit. If the participant is seen within the window of a regularly scheduled visit, all measures for that visit will be collected. If the early termination visit occurs at Weeks 1, 2, 3, 6, 10, 14 or 18, additional safety measures will be obtained (urine sample for biomarkers and pregnancy screen, CES-D and BDI-II if appropriate) since these measures are not scheduled at those visits. The PrimeMD (Patient Health Questionnaire) will be completed at all termination visits.

If the subject is seen outside the regularly scheduled visit window, safety measures will be obtained including: urine sample for TNE and pregnancy screen, BAL, vital signs, CO, adverse events, concomitant medications, return of study product and accountability, Timeline Followback, PrimeMD, and CES-D (BDI-II if appropriate).

The following additional assessments will be completed by all early termination participants using Qualtrics, if not part of the usual measures scheduled for the visit:

- 1) Stages of Change
- 2) Cigarette Evaluation Scale - Experimental
- 3) Drug Effects/Liking
- 4) Perceived Health Risk Study – Experimental
- 5) CES-D
- 6) Study Evaluation
- 7) End of Study

At the time of the early termination visit, willing participants will be scheduled for a Week 20 visit. This visit is necessary to collect primary and secondary data points as well as safety information on all randomized participants for the intent-to-treat analysis.

Study Completion:

Any Adverse Event Forms that remain open from the last study session will be discussed and closed. Once a participant has completed all study procedures and all open events have been closed, the Site Leader will review the participant's binder and sign a form indicating study completion for that participant.

Study Debriefing:

After data collection from all participants is complete, participants will be mailed a letter telling them which condition they were randomized into and the results of the study thus far.

Data Storage:

Data will be stored locally at each site and at the University of Minnesota Masonic Cancer Center's Biostatistics Core for at least 7 years after study completion.

Participant Compensation:

Participants will receive \$25 for completing the screening, regardless of enrollment as long as they pass the drug test, breath alcohol test, and CO test. Individuals who do not pass these tests will be dismissed from the screening visit, except in the event they can produce a prescription for the medication that caused them to fail the drug test. Marijuana use will be tested, but a positive result will not be an exclusionary criterion. The dismissed subjects will be paid transportation costs.

Screening Session: \$25

Shorter visits –Experimental Weeks 1, 2, 3, 6, 10, 14, 18: \$20 per visit for a total of \$140

Longer (biomarker collections) visits - Baseline 91 and 00, Experimental Weeks, 4, 8, 12, 16 and 20: \$30 per visit for a total of \$210

Follow-Up Week 24: \$25

Travel reimbursement (16 visits): \$10 per visit, for a total of \$160 [Note: differences may occur across sites]

To facilitate compliance with the daily IVR calls subjects will earn \$1 per day for the call through week 21: up to \$161 and an IVR diary bonus for completing all calls at \$10 per week through week 21: up to \$230.

To reduce excessive use of cigarettes due to dispensing a weekly supply of 100% over the baseline smoking rate, any unopened packs of study cigarettes returned at each visit will be reimbursed at the following rate: $\geq 25\%$ of returned unopened packs will receive \$5.00; $< 25\%$ returned packs will not receive the return credit.

Total compensation for visits and maximum for IVR and returned pack credit: \$ 1,011

Monetary incentives will be used to help motivate participants to avoid use of non-study tobacco or non-medicinal nicotine and accurately self-report compliance. Incentives may be contingent on several urine sample results from Weeks 14, 16, 18 and 20. Compliance cannot be assessed across both the gradual and abrupt arms until the 0.03 mg nicotine yield dose is reached at Week 18 in the gradual reduction group. A \$1,000 incentive will be provided to study participants who meet urinary total nicotine equivalents (TNE) cut-offs for all tested specimens that indicate compliance or near compliance with avoidance of non-study products. Urinary TNE levels will be judged to be compliant at levels approximately 10% higher than what would be expected for complete compliance. This level was chosen to allow for variation in individual metabolism and would not penalize very occasional non-study product use.

A separate incentive of \$250 will be provided for participants who have accurate self-report of other tobacco or nicotine use and their urinary TNE levels are consistent with their self-report.

Participants will provide urine samples each week of the study and be told that up to 4 of the 12 urine samples will be randomly selected for testing. In actuality, only the urines from Weeks 18 and 20 and possibly Weeks 14 and 16 will be analyzed. It is important for the participant to believe that samples will be randomly analyzed throughout the study to maximize compliance and accurate self-report for the duration of the study.

Participants are free to discontinue at any time and will receive compensation for the sessions that they complete at the same rate as participants who complete the study. If a subject drops out early they will not be eligible for the compliance incentive or accurate self-report compensation since testing for compliance cannot be completed until Week 18 and 20 samples.

PI WITHDRAWAL OR MONITORING OF PARTICIPANTS

For the participant's protection, participants will be withdrawn immediately from the study if any of the following occur:

- 1) **Cardiovascular disease (CVD) event:** typically includes MI (heart attack), PTCA (angioplasty/stenting), bypass surgery, stroke, peripheral vascular disease (arterial blockages in arms or legs leading to procedure or surgery). Less common CVD

problems would be new cardiac arrhythmias (e.g., new atrial fibrillation) or new valvular disease (e.g., mitral or aortic regurgitation.)

- 2) DVT/PE (deep vein thrombosis/pulmonary embolism, i.e., blood clots in the venous system)
- 3) Suicide Attempt: A participant will be withdrawn if he/she attempts suicide at any time during participation in the study or admits to wanting to attempt suicide.
- 4) Psychiatric Hospitalization: A participant will be withdrawn if he/she is hospitalized for psychiatric reasons at any time during participation in the study.
- 5) Pregnancy: If a participant becomes pregnant during the study, she will be withdrawn. The licensed medical professional will follow-up after delivery to ask questions about the health of the baby.
- 6) Expired breath carbon monoxide increase: A participant will be withdrawn from the study if the average of two consecutive CO readings during the same visit is 100 ppm or greater.
- 7) Marked increase in smoking: A participant will be withdrawn from the study if he/she meets **BOTH** of the following criteria for two consecutive weeks
 - a) Cigarette per day increase: The average CPD increases by more than 100% from the average CPD during baseline.
 - b) Expired breath carbon monoxide increase: If the average of two consecutive CO measurements in the same visit is
 - i. CO is greater than 50 ppm if CO at Baseline 1 is <20 ppm
 - ii. CO is greater than 60 ppm if CO at Baseline 1 is 20 - 34 ppm
 - iii. CO is greater than 70 ppm if CO at Baseline 1 is 35 - 49 ppm
 - iv. CO is greater than 80 ppm if CO at Baseline 1 is 50 - 64 ppm
 - v. CO is greater than 90 ppm if CO at Baseline 1 is 65 - 80 ppm

The following will be monitored and can lead to the participant being withdrawn by the Site Leader or Licensed Medical Professional:

- 1) Cigarettes per day increase: Continued participation will be evaluated by the Site Leader if the average number of cigarettes per day (CPD) increases by more than 100% from the average CPD during baseline as determined by CPD on the Timeline Follow Back at Baseline 2.
- 2) Blood pressure (BP) or heart rate (HR) changes: If any of the following occur post-enrollment: 1) BP is at or above 160/100 or below 90/50, or 2) HR is at or above 105 bpm or below 45 bpm, a manual blood pressure and heart rate measurement will be taken after 10 minutes have passed. If the manual reading is still at or above 160/100 for blood pressure or 105 bpm for heart rate, a 'Blood Pressure and Heart Rate Symptom Checklist' will be completed, and the participant will be monitored by the medical professional. If the manual reading is still below 90/50 for blood pressure or 45 bpm for heart rate, a 'Blood Pressure and Heart Rate Symptom Checklist' will be completed. If the participant is symptomatic, an 'Adverse Event Form' will be completed and the participant will be monitored by the medical professional.
- 3) Expired breath Carbon Monoxide increase: If a participant's CO is 1) greater than 50 ppm for participants with CO of less than 20 ppm at Baseline 1; 2) greater than 60 ppm for participants with CO of 20-34 at Baseline 1; 3) greater than 70 ppm for participants with a CO of 35-49 ppm at Baseline 1; 4) greater than 80 ppm for participants with a CO of 50-64 ppm at Baseline 1; 5) greater than 90 ppm for participants with a CO of 65-80 ppm at Baseline 1, another CO reading will be taken after 10 minutes have passed. If the average of the two readings is still out of range the 'Adverse Event Form' will be completed and the participant will be monitored by the medical professional.
- 4) Any hospitalization or debilitation in which participation in the study could be detrimental to the recovery process. This will be self-reported by the participant and will be reviewed by the Site Leader and medical professional to determine whether continued participation in the study is appropriate.
- 5) If a participant is behaving in an inappropriate or threatening manner, admits to lying about eligibility criteria, is participating in other smoking research studies that could affect the primary outcome measures, etc., then the Site Leader can withdraw him/her from the study at the PI's discretion.
- 6) If participation in the study poses an unacceptable risk to the participant (e.g., clinically significant depressive symptoms).
- 7) If a participant fails to attend his/her Baseline 2 Visit (Randomization) within the 21 day allowable visit window, eligibility will be re-assessed according to this person's circumstances.

Thorough information regarding the procedures ensuring subject safety and data integrity are found in the Data Safety and Monitoring Plan.

CERTIFICATE OF CONFIDENTIALITY

To help protect the participant's privacy, a Certificate of Confidentiality from the National Institutes of Health has been obtained. With this certificate, the researchers cannot be forced to disclose the information that may identify the participants, even by a court

subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the participants, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the Federal Food and Drug Administration (FDA).

The Certificate of Confidentiality does not prevent the participant or a member of their family from voluntarily releasing information about his/herself and their involvement in the research. If an insurer, employer or other person obtains the participant's written consent to receive research information, then the researcher may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without consent, information that would identify the individual as a participant of the research project in instances such as evidence of child abuse or a participant's threatened violence to self or others.

OUTCOME VARIABLES

Primary Endpoints

- Toxicant exposure: expired air CO, urine phenanthrene tetrool, and urine mercapturic acid metabolites of a volatile organic chemical (benzene).

Secondary Endpoints

- Cigarettes per day
- Nicotine exposure: cotinine and total nicotine equivalents (TNE)
- Other urine mercapturic acid metabolites of volatile organic chemicals: acrolein (HPMA), propylene oxide (2-HPMA), and crotonaldehyde (HBMA)
- Other toxicant exposures: total NNAL and NNN
- Effect biomarkers: 8-epi-PGF2 α , WBC, and C-reactive protein
- Level of dependence: FTND and WISDM
- Cessation rate and number of 24 hour quit attempts
- Measures of acceptability: retention, compliance (use of non-study cigarettes), and level of discomfort (MNWS, QSU, PANAS, Perceived Stress Scale)

Exploratory Endpoint

- Changes in smoking context: Environmental and Social Influences on Tobacco Use
- Intention to quit: Stages of Change, Contemplation Ladder
- Compensatory smoking: filter analysis
- Cigarette characteristics: Cigarette Evaluation Scale (CES), Drug Effects/Liking Scale, Cigarette Purchase Task
- Perceived risk: Perceived Health Risk Questionnaire

Safety Endpoints

- Potential adverse consequences: Change in mental (CES-D) or physical health (heart rate, blood pressure, Respiratory Questionnaire, Health Changes questionnaire, weight, adverse events)
- Change in alcohol, substance use (TLFB-marijuana use, Alcohol Use Questionnaire, Recreation Drug Use Interview)
- Other tobacco product use
- Adverse event

STATISTICAL APPROACH (see Revised Statistical Analysis Plan)

The main goal of this project is to compare two different approaches to reducing levels of nicotine in cigarettes: an immediate reduction in nicotine content in cigarettes to non-addictive levels or a gradual reduction in nicotine content in cigarettes to non-addictive levels. These two approaches will then be contrasted to a group that continues to smoke cigarettes with nicotine content similar to conventional cigarettes. In addition, we will assess important moderators of product use, biomarkers of exposure and study retention.

Preliminary Analyses:

Baseline covariates will be summarized by treatment group to identify any treatment group imbalances post randomization. This will include demographic characteristics (age, gender, ethnicity, race, education, income, marital status, and employment history), smoking characteristics (CPD and menthol status), and characteristics of nicotine exposure and dependence (total score on FTND, TNE and nicotine metabolite ratio). Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA or Kruskal-Wallis tests depending on whether the ANOVA assumptions are satisfied or not. Suitably transformed variables will be used when necessary. Categorical covariates will be summarized by frequencies and percentages and compared using the Chi-square test or Fisher's exact test, as appropriate.

Primary Endpoint Analysis

The primary endpoint is the toxicant exposure pattern during the 20-week treatment period. The area under the concentration-time curve (AUC) of three main toxicant biomarkers—expired CO, urine phenanthrene tetroal, and urine mercapturic acid metabolites of a volatile organic chemical (benzene)—will first be calculated. Since the concentration of biomarkers is measured at discrete time points, the trapezoidal rule will be used to estimate AUC. Note that this rule also applies when there are intermittent missing observations. In case a missing value is present at week 20, the last available observation will be imputed.

Primary Analysis

Test for normality will be performed for the AUC or transformed AUC data to determine the appropriate transform function form to use in the regression analysis. The AUC for each primary endpoint will be summarized by group using the mean and standard deviation (or median and interquartile range if skewed). The three treatment groups will be compared using linear regression. We expect the three groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis will only adjust for the corresponding biomarker's exposure level at baseline (for precision). Pairwise comparisons of the least square means among the three treatment conditions based on the linear regression will be performed.

Secondary Analysis

As a secondary analysis, we will complete a multivariable linear regression analysis to account for any baseline imbalances. This analysis will adjust for sex, age and race, along with any other relevant covariates that differ across treatment groups at baseline with a p-value less than 0.20. Finally, we will analyze the value of the biomarkers at each visit using a linear mixed model (64). Fixed effects included in the model will include: treatment group, visit, treatment group by visit interaction, baseline level of the corresponding biomarker and study center. A random intercept for each subject will also be included in the model to account for within subject correlation. A significant group by visit interaction would indicate the time course of response to be different across groups. In order to know whether the group difference occurs at an earlier or a later follow-up time point, we compare the least square means of different groups at each follow-up time point based on the fitted mixed regression model.

Secondary Endpoint Analysis

The secondary endpoints at the end-of-treatment visit will be summarized by treatment group and compared between groups using the same statistical methods as described for the baseline variables in the Preliminary Analyses section. We expect that biomarkers of exposure, as possibly other secondary endpoints, will be skewed and log-transformed for analysis. These variables will be summarized using the geometric mean. The primary analysis will be t-test on log-transformed values or Wilcoxon rank sum test for pairwise comparisons and one-way ANOVA for log-transformed values or Kruskal-Wallis test for the comparison among the three treatment groups. For the other continuous secondary endpoints, the primary analysis will be t-test for the pairwise comparisons and the one-way ANOVA for the overall comparison. For continuous endpoints, we will also complete an adjusted analysis using linear regression, adjusting for these variables' baseline values, participants' age, sex and race, along with any other relevant covariates that differ across treatment groups at baseline with a p-value less than 0.20. Finally, a linear mixed model analysis described in the previous section will also be completed for secondary endpoints measured at multiple visits.

The cessation outcome will be summarized by treatment group using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with the cessation outcome.

The retention rate at the end-of-treatment visit, as a measure of acceptability, will be summarized by treatment group using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with the retention. We will also use the Kaplan-Meier method to estimate the retention rate with the time-to-drop-out data and use log-rank test to compare time-varying retention rates. Cox regressions will also be used to identify factors associated with retention. Some candidate factors include subjective responses to products and measures such as Drug Effects/Liking Questionnaire, Perceived Health Risk, extent of willingness to pay for the product, Withdrawal Question, Smoking Urges; gender, race, baseline degree of dependence and baseline nicotine metabolism phenotype.

Exploratory Endpoint Analysis

Our exploratory endpoints will be summarized by treatment group. Categorical variables will be summarized using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. Continuous variables will be summarized using mean, standard deviation, median, and range and compared by F-test or t-test, as appropriate. A secondary analysis using linear or generalized linear regression model will be completed to adjust for the corresponding baseline value, sex, age and race, along with any other relevant covariates that differ across treatment groups at baseline with a p-value less than 0.20.

Subgroup Analyses

Subgroup analyses will play an important role in understanding the effect of nicotine reduction for different subpopulations. Subgroup analyses will follow the same approach described for the primary, secondary, and exploratory endpoints.

Formal testing of the interaction between subgroup and treatment effect is not the primary concern of our subgroup analyses. Instead, subgroup analyses will provide information about the consistency of the treatment effect across subgroups, which will provide supplementary information for full understanding of the relationship between the studied interventions and the study endpoints.

The primary subgroups of interest are defined by sex (men vs. women), race (white vs. non-white), nicotine metabolite ratio (high vs. low, based on median value), menthol status (menthol vs. non-menthol), and level of dependence (high vs. low, determined by baseline FTND). Given equal recruitment of males and females, we will be able to detect an effect size of 0.4 for a reduction group vs. control and of size 0.3 between the two reduction groups, for analyses focused on a single sex, with an 80% power. Similar effect sizes will be detected for a single race group (or a single menthol status group or dependence group) if the two subgroups have similar sizes.

Missing Data Analysis

Every effort will be made to limit the amount of missing data in this trial. Study participants will be incentivized to attend study sessions and complete the daily IVR as detailed in the study protocol (Participate Compensation subsection). However, some level of missing data is inevitable in a study of this kind. In response, we will complete a sensitivity analysis for the primary and secondary endpoints in order to evaluate the robustness of our conclusions to missing data.

First, we will compare subjects with and without missing data in order to identify baseline covariates (including the treatment group variable) associated with missing data. We will then conduct a sensitivity analysis of primary and secondary endpoints using multiple imputation where missing values are imputed using regression models developed from baseline covariates (66). If treatment group is associated with missing, we will conduct multiple imputation for each treatment group separately (67). Note that for the primary endpoints which are measured as AUC, the AUC based on the imputed data will be calculated using the same trapezoidal rule as for the data with missing values.

When there is no differential retention (or drop-out) rate (per the result of the retention analysis described previously), we will conduct additional sensitivity analysis for the cessation outcome, where missing smoking status is recoded as smoking. This will serve as a "worse-case scenario" as all subjects are smokers at baseline. The results of these analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

POWER ANALYSIS

Our study is powered to detect a significant difference between the immediate reduction and the gradual reduction condition and between each of the two reduction conditions with the control condition. Power analyses are based on two-sided, two-sample t-tests at the 0.0055 level to account for the multiple comparisons (3 pairwise comparisons between the 3 conditions for each of the three biomarkers; $3 \times 3 \times 0.0055 = 0.05$). Based on a 30% attrition rate over the 20 week period, a sample size of 1250 subjects in total (500, 500, and 250 for the immediate reduction, gradual reduction, and control condition, respectively) will achieve 80% power to detect an effect size of 0.5 between a reduction group and the control group and an effect size of 0.4 between the two reduction groups. These are comparable or less than the treatment effect of reduced nicotine content (RNC) vs. control at week 26 observed by Benowitz et al. (65) for biomarkers such as cotinine (effect size ~ 0.8) and nicotine (effect size ~ 1.0). PASS version 11.0.2© (NCSS, LLC) was used for power calculation.

We recognize the possible existence of the correlation between the three pairwise tests among the three treatment arms and the fact that Bonferroni method does not take into account such correlation. However, when such correlation exists, the powers calculated based on the Bonferroni method will be less (i.e. conservative) than the actual power. In other words, the actual power will be as large as or larger than the power estimates described above.

SUBJECT IDENTIFIER

Study Identifier

B = Project 2

The subject identifier is an alpha-numeric combination. Example: B001 would be Minnesota's first subject.

Site Identifier

B = UMN

D = Johns Hopkins

E = Penn

F = Duke

G = MD Anderson

H = UCSF

I = UMN-D

J= Moffitt Cancer Center

K= Mayo Clinic Scottsdale

L = Oregon Research Institute

Visit Numbers:

92= Screen

91= Baseline 1

00= Baseline 2 – RANDOMIZATION VISIT

01= Week 1 clinic visit

02= Week 2 clinic visit

03= Week 3 clinic visit

04= Week 4 clinic visit

06= Week 6 clinic visit

08= Week 8 clinic visit

10= Week 10 clinic visit

12= Week 12 clinic visit

14= Week 14 clinic visit

16= Week 16 clinic visit

18= Week 18 clinic visit

20= Week 20 clinic visit

24= Week 24 follow-up visit

99= UNSCHEDULED/ADDITIONAL visit

TABLE 1: PROCEDURES BY VISIT

Assessment Name	Platform	Screen	BL-1	BL00	Weeks 1-3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20	Follow-up 24	Early Term
Telephone Recruitment Questionnaire	REDCap	X														
VISIT ASSESSMENTS																
Weight (Height at screen)	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test (if applicable)	Paper and REDCap			X		X		X		X		X		X		X
Heart Rate & BP	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
NicAlert (if low CO)	Paper and REDCap	X														
Breath Alcohol Test	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Toxicology	Paper and REDCap	X												X		
Concomitant Medications	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Health Changes Questionnaire	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events Assessment	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Product Accountability Log	Paper and REDCap			X	X	X	X	X	X	X	X	X	X	X		X
Product Procedures & Compliance Review Sessions	Paper		X	X	X	X	X	X	X	X	X	X	X	X		
Intention to Quit Log	Paper and REDCap			X	X	X	X	X	X	X	X	X	X	X		
End of Visit Evaluation	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Evaluation Questionnaire	Qualtrics													X		X
SCREENING QUESTIONNAIRES																
Tobacco Use History and Exposure	Paper and REDCap	X														
Smoking Cessation Therapy	Paper and REDCap	X														
Identifying Information	Paper and Access	X														
Demographics for Registration	Paper and REDCap	X														
Demographics	Qualtrics	X														
Brief Medical History	Paper and REDCap	X														
Prime MD (Follow-up if applicable)	Paper and REDCap	X												X		X
CES-D	Qualtrics	X					X		X		X		X		X	X
Beck Depression Inventory (BDI-II)(if CES-D positive)	Paper (source only)	X					X		X		X		X		X	X
Beck Depression Inventory (BDI-II) (if monitoring needed)	Paper (Source only)		X	X	X	X	X	X	X	X	X	X	X	X		X
Intl. Neuropsychiatric Interview (MINI) Suicide Subscale	Paper (Source only)	X														
Short Michigan Alcohol Screening Test (SMAST)	Qualtrics	X												X		
Drug Abuse Screening Test (DAST-brief)	Qualtrics	X												X		

TABLE 1: PROCEDURES BY VISIT

Assessment Name	Platform	Screen	BL-1	BL00	Weeks 1-3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20	Follow-up 24	Early Term
Eligibility Checklist	Paper and REDCap	X														
Alcohol Use Questionnaire 12 mo & 1 mo	Qualtrics	X														
Drug Use Questionnaire - 12 month & 1 mo	Paper and REDCap	X														
TOBACCO USE MEASURES																
Daily Tobacco Diary (IVR)	IVR	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Timeline Follow-back	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADVERSE CONSEQUENCES																
MNWS	Qualtrics		X	X	X	X	X	X	X	X	X	X	X	X		X ^b
Questionnaire on Smoking Urges Usual cigs, Baseline	Qualtrics		X	X												
Questionnaire on Smoking Urges Usual cigs	Qualtrics				X	X	X	X	X	X	X	X	X	X		X ^b
Questionnaire on Smoking Urges Study cigs	Qualtrics				X	X	X	X	X	X	X	X	X	X		X ^b
PANAS	Qualtrics		X			X		X		X		X		X		
Alcohol Use Questionnaire 1 month	Qualtrics			X		X		X		X		X		X		
Drug Use Questionnaire - 1 month	Paper and REDCap			X		X		X		X		X		X		
Perceived Stress Scale-4 Item Scale	Qualtrics			X		X		X		X		X		X		
Respiratory Health Questionnaire	Qualtrics		X	X	X	X	X	X	X	X	X	X	X	X		X ^b
DEPENDENCE MEASURES																
FTND	Qualtrics	X				X		X		X		X		X	X	
WISDM - brief	Qualtrics		X			X		X		X		X		X		
Stages of Change	Qualtrics	X												X	X	X
ACCEPTABILITY MEASURES																
Cigarette Evaluation Scale Usual brand	Qualtrics		X	X											X	
Cigarette Evaluation Scale Study cigs	Qualtrics				X	X		X		X		X		X		X
Drug Effects/Liking Scale Usual cigs	Qualtrics			X												
Drug Effects/Liking Scale Study cigs	Qualtrics				X	X		X		X		X		X		X
Perceived Health Risk - Baseline-Usual cigs	Qualtrics		X													
Perceived Health Risk - Baseline-No tobacco	Qualtrics		X													
Perceived Health Risk - Study cigs	Qualtrics					X		X		X		X		X		X
Cigarette Purchase Task Usual cigs	Qualtrics			X		X		X		X		X		X		
Cigarette Purchase Task study cigs	Qualtrics					X		X		X		X		X		
End of Study Questionnaire	Qualtrics													X		X
OTHER																
Environmental & Social Influences on Tobacco Use	Qualtrics			X										X		

TABLE 1: PROCEDURES BY VISIT

Assessment Name	Platform	Screen	BL-1	BL00	Weeks 1-3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20	Follow-up 24	Early Term
Saliva specimen for possible future DNA analysis	Biosample Database & Excel ^a			X												
EXPOSURE BIOMARKER AND RISK FACTORS																
Carbon Monoxide	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Total Nicotine Equivalents (urine)	Biosample Database & Excel ^a			X		X		X		X		X		X	X	X
Total NNAL & NNN (urine)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
Mercapturic acids of acrolein, benzene, 1,3-butadiene, crotonaldehyde, and ethylene oxide (urine)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
8-epi-PGF (urine)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
Phenanthrene Tetroal (urine)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
Spot Urine for compliance testing	Biosample Database & Excel ^a				X	X	X	X	X	X	X	X	X	X		X ^c
Nicotine Metabolite Ratio (serum)	Biosample Database & Excel ^a			X												
CVD Risk Factor-Hemogram (whole blood)	REDCap (results only)			X		X		X		X		X		X		X ^c
CVD Risk Factor--C-Reactive Protein (serum)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
Oral Cell Collection (UMN-TC & UMN-D only)	Biosample Database & Excel ^a			X		X		X		X		X		X		X ^c
Cigarette Butt Analysis (CDC) (only UMN-TC, UCSF & Moffitt)	REDCap			X		X		X		X		X		X		X ^c

^a Specimen label scanned before shipment

^b Administered if early termination is within visit window

^c Samples may be used if subject was on study cigarettes at early termination visit

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PROTOCOL REVISION HISTORY

Protocol Version Date	Revision Summary
5/1/2014	Project 2 Study Protocol (Investigative Plan) submitted to sites
6/10/2014	<p>REVISED CENIC P2 Protocol Multiple corrections/ clarifications The primary changes include:</p> <ul style="list-style-type: none"> • Monthly pregnancy testing per FDA; • Compliance bonus with a semi-bogus pipeline indicating random urine checks to enhance adherence to study products; • Subjects will receive a small incentive to bring in unopened packs of cigarettes to reduce opening multiple packs simultaneously or sharing cigarettes; • Substituting caTissue to track samples with a database developed by Oncology Medical Informatics; • IVR will have two weeks of baseline calls rather than one week; • Sample shipping will be upon request rather than quarterly; • Oral cells will not be collected at all sites, only at Minnesota sites since N of 250 is sufficient.
7/16/14	<p>Changes include:</p> <ul style="list-style-type: none"> • Addition to "Potential Risks of Participation: <ul style="list-style-type: none"> • IRB request to add Ectopic pregnancy and macular degeneration; • FDA recommendation to add a statement that the study cigarettes are made from GMO tobacco; • Heart Rate reduced from 115 bpm to 105 bpm for eligibility per FDA recommendation; • End of study bonus of \$75 for on time visits during the study per request from sites that experienced challenges with subject attendance (subsequently removed); • Updated statistical section per FDA request for further detail regarding secondary and missing data; • Subset of 3 sites were implemented for collection of spent cigarette butts (UMN-TC, Moffitt, UCSF) to assess intensity of smoking; • Added the following exclusion medications because they have been shown to interact with nicotine or tobacco smoke. Nicotine reduction or smoking cessation may impact the metabolism of these medications. <ul style="list-style-type: none"> a. Bendamustine (Treanda) b. Clopidogrel (Plavix) c. Clozapine (Clozaril, FazaClo) d. Erlotinib (Tarceva) e. Flecainide (Tambocor) f. Fluvoxamine (Luvox) g. Irinotecan (Camptosar) h. Olanzapine (Zyprexa) i. Ropinirole (Requip) j. Tacrine (Cognex) a.k. Theophylline (Theo Dur, etc.) • Change pack return credit to improve tracking of the study cigarettes.
9/15/14	<p>Changes include:</p> <ul style="list-style-type: none"> • Monetary Incentive \$1000 for only smoking study cigarettes (or abstinence) or \$250 accurate self-report to enhance accurate reporting of non-study cigarette/tobacco usage due to results from a recently completed study that demonstrated that 75% were not compliant with using only study cigarettes; • Visit payment reduced for short and long visit from \$40 and \$60 to \$20 and \$30 respectively (transportation of \$10 per visit was constant) to accommodate bonus payment; • Additional risk added for medication and smoking interaction; • 1st morning urinary void is required at baseline and spot urine could not be substituted in order to obtain more accurate assessment of baseline biomarkers; • Minor editorial corrections
2/11/2015	<p>Changes include:</p> <ul style="list-style-type: none"> • Additional exclusion criteria (page 14):

	<ul style="list-style-type: none"> • Previous use of VLNC <3yrs are ineligible in order to minimize enrollment of individuals who had previous experience with VLNC cigarettes so that the blind would be broken; • Unstable living condition to avoid misplacement or misuse of study cigarettes; • Early termination visit procedures (page 26) clarified; • Wording changed to ensure that the participant more clearly understands that the compliance incentive is for avoiding non-study tobacco products or e-cigarettes, not for smoking study cigarettes. (page 27; per NIDA request); • Minor corrections (e.g. typos) • Removed Access as the database we are using (page 11); • TNE assay used instead of cotinine for more precise measure of nicotine exposure;
6/1/2015	<p>Changes include:</p> <ul style="list-style-type: none"> • Request that participants who terminate early from the study attend an End of Trial (Week 20) visit to determine tobacco use and medical status for the Intent-to-Treat analysis per DSMB recommendation. • If a subject's week 24 biomarkers are substantially elevated above baseline, subjects may be requested provide an additional urine sample for a safety check.
4/7/2017	<p>Statistical analysis section was updated in the Statistical Analysis Plan to reflect revised statistical methods. See Statistical Analysis Plan dated 4/7/2017 for these revisions.</p>

**Statistical Analysis Plan
CENIC, Project 2
7/14/2014**

Modified 4/7/17

- Primary outcome was changed from benzene to acrolein (we substituted 3HPMA for benzene because benzene is affected by GST-T1 genotype);
- We have added another mercapturic acid metabolite of a volatile organic compound, acrylonitrile, (CEMA);
- Greater description has been provided for the of analysis of retention because of the recommendations made by our Data Safety and Monitoring Board to conduct a week 20 follow-up of subjects who dropped out. Completers have now been defined as missing one or less visits that involved dose changes within the gradual reduction group (weeks 0, 4, 8, 12 and 16), but applied to both conditions.
- Comparisons between subjects who have missing values vs. no missing values have been eliminated because the majority of subjects have at least one missing value in this lengthy protocol. Instead, we added additional analysis of completers vs. non-completers and also an analysis of those who were compliant based on Total Nicotine Equivalent values at Week 18 and 20;
- We have eliminated the interim analysis as discussed with our investigators and the FDA.

Modified 12/20/17

- The estimated effect sizes for the overall group analysis (in Section 3.2) and the subgroup analyses (in Section 6.6) were mistakenly switched in the previous versions of SAP. They have been corrected in this version.
- In the previous versions of SAP, we proposed no p-value adjustment for secondary or other endpoints. In the revised SAP, we propose to use a more stringent cutpoint, 0.0167 to account for the multiple group comparisons within each endpoint.
- In the previous version, we adjusted different sets of covariates in the analyses for different endpoints. In the revised SAP, we propose to adjust the same set of covariates (the baseline level of the outcome variable, study center, and any covariates which are different across treatment groups at $p < 0.20$) in all adjusted regression analyses, and for the minimally adjusted models, we propose to adjust only the baseline level of the outcome variables (whenever available).

Modified 5/28/18

Major Changes

- We have changed the primary missing data method to the multiple imputation method. We have added an imputation method using baseline value for dropouts as a secondary missing data method. We believe that this method would provide the most conservative estimate of differences between gradual vs. immediate. This method assumes that all smokers would resort to illicit trade cigarettes and additionally, fewer differences would be observed between the gradual and immediate reduction group due to the higher drop-outs in the immediate reduction group, thereby biasing against this group. Last observation carried forward method is now a secondary missing data method, which is likely a more liberal (i.e., anti-conservative) method than the MI and baseline value imputation. That is, this approach is likely to lead to greater differences between the gradual and immediate reduction approach, particularly if the participants in the gradual reduction approach dropped out earlier and demonstrated higher exposure levels.
- In the previous version of SAP, the p-value cutpoint for the significance of secondary endpoints was only adjusted for multiple group comparisons within each endpoint, but not adjusted for multiple secondary endpoints (29 in total). In this version, we propose to use a more stringent cutpoint, 0.00057 ($= 0.05/29$ endpoints/3 pairwise comparisons) to maintain the secondary endpoints' family-wise type I error at 0.05.

Minor Changes or Clarifications

- Two reference on effect size (Section 3.2) have been added. According to Sawilowsky (2009), the effect sizes (0.3 and 0.4) used for sample size calculation of this study were small to medium.
- In the revision, we have clarified that the AUC is scaled by time (i.e., time-scaled AUC), and hence the unit of AUC is the same as the unit of its respective exposure variable. We have added rationales for using AUC as the primary endpoints in Section 6.3.
- In the revision, we have added more details about the multiple imputation method (Section 6.8.1) and repeated measures analysis (Section 6.4), which were not included in the previous versions.

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1. Introduction

This document will serve as the Statistical Analysis Plan for CENIC Project 2, entitled “Strategies for Reducing Nicotine Content in Cigarettes”. This document describes the planned statistical analysis for evaluating the product use patterns, biomarkers of exposure, and subjective or other behavioral responses between smokers assigned to immediate reduction vs. gradual reduction in nicotine content in cigarettes to non-addictive levels and both groups will be compared to conventional yield cigarette group. Details for the proposed analysis of the primary, secondary and other endpoints are provided.

2. Trial Objectives

The main goal of this project is to compare two different approaches to reducing levels of nicotine in cigarettes: an immediate reduction in nicotine content in cigarettes to non-addictive levels or a gradual reduction in nicotine content in cigarettes to non-addictive levels. These two approaches will then be contrasted to a group that continues to smoke cigarettes with nicotine content similar to conventional cigarettes. In addition, we will also determine important moderators of product use and exposure and study retention.

3. Trial Design

This is a randomized, multi-center, double-blind study design. 1250 subjects will be recruited from 10 academic institutions and randomized to one of three conditions:

1) immediate reduction to 0.03 mg nicotine yield or very low nicotine content (VLNC) cigarettes (n=500 participants); 2) gradual reduction in nicotine content cigarettes, with each reduction occurring monthly, i.e., weeks 4, 8, 12, and 16 (referred to as dose changing visits hereinafter) (n=500 participants); and 3) experimental cigarettes with conventional levels of nicotine (0.8 mg nicotine yield; n=250 participants). The three conditions will be referred as the immediate reduction, gradual reduction, and control condition, respectively, hereinafter.

Participants in each condition will be assigned cigarettes that match that menthol preference. Participants will be blinded to the dose and provided the experimental cigarettes for a period of 5 months. Participants will complete questionnaires on demographics, smoking and health history, and drug and alcohol use history at baseline. Throughout the experimental phase, participants will record their use of tobacco/nicotine products and complete questionnaires on mood and responses to the study tobacco product. Biomarker samples will be analyzed for exposure levels of nicotine and tobacco-related toxicants.

3.1. Randomization

Subjects will be randomized with a 2:2:1 ratio to the three studied conditions described above, respectively. Randomization will be stratified by study center. We will use block randomization with random blocks of size five and ten to improve balance. We anticipate no or less than minimal block effects in our data because we do not expect the characteristics or responses for a participant to change according to their entry time into the study. We will estimate the intrablock correlation coefficient (Matts and Lachin, 1988) for the primary endpoints for the overall sample and for each center. A Bootstrap method, with repeatedly sampled blocks, will be used to obtain the 95% confidence interval (CI) of the estimated intrablock correlation. A 95% CI which does not cover 0 would indicate a significant non-zero intrablock correlation, in which case, we will apply mixed effects models by adding a random effect for blocks to appropriately account for the blocking effect.

3.2. Sample Size

Our primary analysis will focus on the toxic exposure over the 20-week treatment period. The area under the concentration-time curve (AUC) of three main toxicant biomarkers—expired CO, urine phenanthrene tetroal, and urine mercapturic acid metabolites of a volatile organic chemical (acrolein)—will be compared between the three treatment groups. Our study is powered to detect a significant difference between the immediate reduction and the gradual reduction condition and between each of the two reduction conditions with the control condition. Power analyses are based on two-sided, two-sample t-tests at the 0.0055 level to account for the multiple comparisons (3 pairwise comparisons among the 3 conditions for each of the three biomarkers; $3 \times 3 \times 0.0055 = 0.05$). Based on a 30% attrition rate over the 20 week period, a sample size of 1250 subjects in total (500, 500, and 250 for the immediate reduction, gradual reduction, and control condition, respectively) will ensure us to achieve 80% power to detect an effect size (Cohen, 1988) of 0.4 between a reduction group and the control group and an effect size of 0.3 between the two reduction groups. These are comparable to or less than the treatment effect of reduced nicotine content (RNC) vs. control at week 26 observed by Benowitz et al. (2012) for biomarkers such as cotinine (effect size ~0.8) and nicotine (effect size ~1.0). PASS version 11.0.2© (NCSS, LLC) was used for power calculation. According to Cohen (1988) and Sawilowsky (2009), the effect sizes (0.3 and 0.4) used for sample size calculation are between small (0.2) and medium (0.5).

We recognize the possible existence of the correlation between the three pairwise tests among the three treatment arms and the fact that Bonferroni method does not take into account such correlation. However, when such correlation exists, the powers calculated based on the Bonferroni method could only be less (i.e. conservative) than the actual power. In other words, the actual power could be as large as or bigger than the powers that we estimated.

4. Study Populations

4.1. Intent-to-Treat

The primary analysis of all endpoints will adhere to the intent-to-treat principle. Under this principle, all randomized subjects will be included in the analysis in the group to which they were randomized regardless of protocol violations and compliance to treatment assignment. Additional analysis will be conducted among completers (defined as completing week 20 visit and not skipping more than one dose change visit) and among compliant only (defined as TNE value at visit 20 of ≤ 12 nmol/mL with a sensitivity analysis conducted among participants with TNE value at visit 20 of ≤ 6.41 nmol/mL).

4.2. Definition of Sub-Group Population in Different Analyses

We intend to complete pre-planned subgroup analyses by sex, race (white vs. other), menthol status, level of dependence (high vs. low, determined by baseline FTND), and nicotine metabolic ratio (high vs. low, determined by the median value). Subgroup analysis will allow us to evaluate the consistency of the effect of immediate reduction vs. gradual reduction and the effect of both reduction conditions compared to the control condition, across important subgroups.

5. Trial Endpoints

5.1. Primary Endpoints

- Toxicant exposure: expired air CO, urine phenanthrene tetroal, and urine mercapturic acid metabolites of a volatile organic chemical (acrolein 3HPMA).

5.2. Secondary Endpoints (29 in total)

- Cigarettes per day (total CPD, study CPD, and non-study CPD)
- Nicotine exposure: cotinine and total nicotine equivalents (TNE)
- Other urine mercapturic acid metabolites of volatile organic chemicals: benzene (SPMA), propylene oxide (2-HPMA), acrylonitrile (CEMA), and crotonaldehyde (HBMA)
- Other toxicant exposures: total NNAL and NNN
- Effect biomarkers: 8-epi-PGF2 α , WBC, and C-reactive protein
- Level of dependence: FTND (FTND with CPD and FTND without CPD) and WISDM (total score, primary motive score, and secondary motive score)
- Rate of 24 hour smoking abstinence (calculated as the percent of people who made at least one 24 hour smoking abstinence) and number of days of 24 hour abstinence during weeks 0-20
- Measures of acceptability: retention, compliance (use of non-study cigarettes), and level of discomfort (MNWS, QSU [QSU Factor 1 and QSU Factor 2], PANAS [PANAS positive effect score and negative effect score], Perceived Stress Scale [total score])

5.3. Exploratory Endpoint

- Changes in smoking context: Environmental and Social Influences on Tobacco Use
- Intention to quit: Stages of Change, Contemplation Ladder
- Compensatory smoking: filter analysis (if provided by CDC)
- Cigarette characteristics: Cigarette Evaluation Scale (CES), Drug Effects/Liking Scale, Cigarette Purchase Task
- Perceived risk: Perceived Health Risk Questionnaire

5.4. Safety Endpoints

- Potential adverse consequences: Change in mental (CESD) or physical health (heart rate, blood pressure, Respiratory Questionnaire, Health Changes questionnaire, weight, adverse events)
- Change in alcohol, substance use (TLFB-marijuana use, Alcohol Use Questionnaire, Recreation Drug Use Interview)
- Other tobacco product use
- Adverse events

6. Statistical Analysis

6.1. General Approach

All statistical analyses will be performed using SAS (version 9.4) or R (version 3.3.3). All statistical tests will be two-tailed. A Bonferroni multiple-comparisons adjustment will be used to account for multiple comparisons among the 3 studied conditions for each of the three primary endpoints. Therefore, p-values less than 0.0055 ($=0.05/3$ primary endpoints/3 pairwise comparisons) for between treatment comparisons will be considered significant. For secondary endpoints, p-values less than 0.00057 ($=0.05/29$ secondary endpoints/3 pairwise comparisons) will be considered significant to maintain a family-wise type-I error rate for secondary endpoints at 0.05. For exploratory and other endpoints, p-values less than 0.0167 ($=0.05/3$ pairwise comparisons) will be considered significant to account for the multiple group comparisons within each

endpoint. All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below. Model diagnosis will be routinely performed for all analyses.

6.2. Describing the Study Population

Baseline covariates will be summarized by treatment group to identify any treatment group imbalances post randomization. This will include demographic characteristics (age, gender, ethnicity, race, education, income, marital status, and employment history), smoking characteristics (CPD and menthol status), and characteristics of nicotine exposure and dependence (total score on FTND, TNE and nicotine metabolite ratio). Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA or Kruskal-Wallis tests depending on whether the ANOVA assumptions are satisfied or not. Suitably transformed variables will be used when necessary. Categorical covariates will be summarized by frequencies and percentages and compared using the Chi-square test or Fisher's exact test, as appropriate.

6.3. Primary Endpoint Analysis

The primary endpoint is the toxicant exposure pattern during the 20 week treatment period. The area under the concentration-time curve (AUC) of three main toxicant biomarkers—expired CO, urine phenanthrene tetraol, and urine mercapturic acid metabolites of a volatile organic chemical (acrolein)—will first be calculated. Since the concentration of biomarkers is measured at discrete time points, the trapezoidal rule will be used to estimate AUC. Note that the AUC is scaled by time (i.e., time-scaled AUC), hence, the unit of AUC is the same as the unit of its respective exposure variable.

The rationales for using AUC rather than the 20-week outcome as the primary endpoints are as follows. The intent of this study is to determine the cumulative exposures and smoking behaviors that would occur during the process of implementing each of these approaches if the FDA were to institute a standard to reduce all cigarettes to minimally addictive levels rather than to determine the exposures that would occur at the end of each of these approaches. For example, in the recent FDA's Advanced Notice of Proposed Rulemaking, a specific question that was asked was whether or not an immediate or a gradual approach to nicotine reduction should be implemented. To determine the public health benefit or risk of each of these approaches, the extent of exposures from a gradual nicotine reduction approach that would last over several years versus an immediate reduction approach and possible unintended consequences of each of these approaches must be determined. The effects at the end of each of these approaches would only provide a limited picture. Therefore, we will use the AUC analysis as primary and the week 20 analyses as secondary (per review from the FDA).

6.3.1. Primary Analysis

Test for normality will be performed for the AUC or transformed AUC data to determine the appropriate transform function form to use in the regression analysis. The AUC for each primary endpoint will be summarized by group using the mean and standard deviation (or median and interquartile range if skewed). The three treatment groups will be compared using linear regression for AUC or log AUC if AUC is skewed. For AUC, the treatment effect will be presented as mean difference in AUC; for log AUC, the treatment effect will be presented as ratio of geometric means, which is calculated as the exponential of the mean difference in log AUC. We expect the three groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis will only adjust for the corresponding biomarker's exposure level at baseline (for precision). Pairwise comparisons of the least square means among the three treatment conditions based on the linear regression will be performed.

6.3.2. Secondary Analysis

As a secondary analysis, we will complete a multivariable linear regression analysis to account for any baseline imbalances. This analysis will adjust for the baseline level of the outcome variable, study center, and any covariates from the list of covariates provided in Section 6.2 that differ across treatment groups at baseline with a p-value less than 0.20.

Finally, we will analyze the value of the biomarkers at each visit using a linear mixed model (Verbeke and Molenberghs, 2000). Fixed effects included in the model will include: treatment group, visit, treatment group by visit interaction, and the baseline level of the corresponding biomarker. A random intercept for each subject will also be included in the model to account for within subject correlation. A significant group by visit interaction would indicate the time course of response to be different across groups. In order to know whether the group difference occurs at earlier or later time points, we compare the least square means of different groups at each visit time point based on the fitted mixed regression model. A second linear mixed model will be performed by additionally adjusting for study center and any covariates from the list of covariates provided in Section 6.2 that differ across treatment groups at baseline with a p-value less than 0.20.

6.4. Secondary Endpoint Analysis

The secondary endpoints at the end-of-treatment visit will be summarized by treatment group and compared between groups using the same statistical methods as described for the baseline variables in Section 6.2.

We expect that biomarkers of exposure, and possibly other secondary endpoints, will be skewed and log-transformed for analysis. These variables will be summarized using the geometric mean. The primary analysis will be linear regression adjusting for the corresponding baseline level. For continuous endpoints, we will also complete an adjusted analysis using a similar linear regression as described in Section 6.3.2. Finally, a linear mixed model analysis similar to the model described in Section 6.3.2 will also be completed for secondary endpoints measured at multiple visits.

The cessation outcomes will be summarized by treatment group using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with the cessation outcome.

The retention rate at the end-of-treatment visit (defined as percent of people completing week 20 visit and missing one or less dose changing visits), as a measure of acceptability, will be summarized by treatment group using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with the retention. We will also use the Kaplan-Meier method to estimate the retention rate with the time-to-drop-out data and use log-rank test to compare time-varying retention rates. Cox regressions will also be used to identify factors associated with retention. Some candidate factors include subjective responses to products and measures such as Drug Effects/Liking Questionnaire, CES, Perceived Health Risk, extent of willingness to pay for the product, Withdrawal Question, Smoking Urges; gender, race, baseline degree of dependence and baseline nicotine metabolism phenotype.

6.5. Exploratory Endpoint Analysis

Our exploratory endpoints, listed in Section 5.3, will be summarized by treatment group. Categorical variables will be summarized using frequencies and percentages and compared by the chi-square test or Fisher's exact test, as appropriate. Continuous variables will be summarized using mean, standard deviation, median, and range and compared by F-test or t-test, as appropriate.

A secondary analysis using linear or generalized linear regression model will be completed to adjust for the corresponding baseline value, sex, age and race, along with any other covariates from the list of covariates provided in Section 6.2 that differ across treatment groups at baseline with a p-value less than 0.20.

6.6. Subgroup Analyses

Subgroup analyses will play an important role in understanding the effect of nicotine reduction for different subpopulations. Subgroup analyses will follow the same approach described in Sections 6.3 – 6.5 for the primary, secondary, and exploratory endpoints.

Formal testing of the interaction between subgroup and treatment effect is not the primary concern of our subgroup analyses. Instead, subgroup analyses will provide information about the consistency of the treatment effect across subgroups, which will provide supplementary information for full understanding of the relationship between the studied interventions and the study endpoints.

The primary subgroups of interest are defined by sex (men vs. women), race (white vs. non-white), menthol status (menthol vs. non-menthol), level of dependence (high vs. low, determined by baseline FTND), and nicotine metabolic ratio (high vs. low, determined by the median value). Given equal recruitment of males and females, we will be able to detect an effect size of 0.5 for a reduction group vs. control and of size 0.4 between the two reduction groups, for analyses focused on a single sex, with an 80% power. Similar effect sizes will be detected for a single race group (or a single menthol status group or dependence group or a single nicotine metabolic ratio group) if the two subgroups have similar sizes.

6.7. Safety

The distinction between the primary/secondary outcomes and safety outcomes is not as clear in this trial as it would be in a typical clinical trial of a novel therapeutic agent. Many outcomes that would typically be considered potential adverse consequences or safety outcomes will be analyzed similarly as the secondary outcomes. For example, the other tobacco product use will be analyzed using similar statistical methods as for the other continuous primary/secondary endpoints. AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared across treatment groups.

6.8. Missing Data

Every effort will be made to limit the amount of missing data in this trial. Study participants will be incentivized to attend study sessions and complete the daily IVR as detailed in the study protocol (Participate Compensation subsection). However, some level of missing data is inevitable in a study of this kind. First, we will compare completers (completing week 20 visit and missing one or less dose change visits) vs. non-completers (not completing week 20 visit or missing greater than one dose change visit) in order to identify baseline covariates (including the treatment group variable) associated with missing data.

6.8.1. Primary Analysis for Missing Data

The primary missing data method for continuous variables will be the multiple imputation with the Markov Chain Monte Carlo (MCMC) method (Schafer, 1997; Little and Rubin, 2002), carried out in PROC MI in SAS. If the treatment group is associated with missing, we will conduct multiple imputation for each treatment group separately (Molenberghs and Kenward, 2007). Proper transformation such as the logarithmic transformation will be applied to variables in the imputation for variables that are skewed.

For biomarker variables and cigarettes per day variables, the AUC based on the imputed data will be calculated using the trapezoidal rule as for the complete data. For other continuous variables, the imputed Week 20 values will be analyzed. Twenty imputed data will be generated, with the treatment effect being assessed in each imputed data set. An adjusted degrees of freedom for each parameter estimate will be used for the imputed data (Barnard and Rubin, 1999). A final single assessment of treatment arm difference will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS. For the cessation outcome, we will recode missing as smoking.

6.8.2. Sensitivity Analysis

We will also complete a sensitivity analysis for the primary endpoints, by using two different simple imputation methods: the baseline value imputation or last observation carried forward method for dropouts. The trapezoidal rule will be used for intermittent missing. The AUC based on the imputed data will be calculated using the same trapezoidal rule as for the complete data.

The results of these analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

6.9. Interim Analyses

No interim analyses will be conducted.

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