Protocol

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

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Reduced Nicotine Standards for Cigarettes: A Randomized Trial

Appendix B: Supplementary Materials

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PROJECT 1, STUDY 1:

INVESTIGATING THE IMPACT OF NICOTINE USING SPECTRUM CIGARETTES

STUDY PROTOCOL

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Abbreviations

- VLNC: Very low nicotine content
- RNC: Reduced nicotine content
- NNC: Normal nicotine content
- CPD: Cigarettes per day
- CO: Carbon monoxide
- BAL: Breath alcohol levels
- BP: Blood pressure
- HR: Heart rate
- BPM: Beats per minute
- NMR: Nicotine metabolite ratio
- NNN: *N*'-nitrosonornicotine
- NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
- BDI: Beck's Depression Inventory
- FTND: Fagerström Test for Nicotine Dependence
- WISDM: Wisconsin Index of Smoking Dependence Motives
- CESD: Center for Epidemiological Studies Depression Scale
- TLFB: Timeline Follow Back
- MNWS: Minnesota Nicotine Withdrawal Scale
- PANAS: Positive and Negative Affect Scale
- QSU: Questionnaire of Smoking Urges
- CES: Cigarette Evaluation Scale
- PSS: Perceived Stress Scale
- SMAST: Short Michigan Alcohol Screening Test
- DAST: Drug Abuse Screening Test
- CPT: Continuous Performance Task
- IVR: Interactive Voice Response

CENIC Project 1, Study 1 Protocol

Objective:
Project 1, Study 1 will evaluate the relationship between nicotine yield of very low nicotine content cigarettes and cigarettes smoked per day, nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. We will also consider differences between conditions in compliance with product use.

Background Information:
About 5 million people die every year world-wide because of tobacco. The Family Smoking Prevention and Tobacco Control Act (FSPTCA; Congress, 2009) gives the FDA the authority to limit (but not eliminate) the nicotine content of cigarettes. Drastically reducing nicotine content has the potential to reduce cigarette reinforcement and dependence, which in turn may result in less consumption per individual smoker and a greater likelihood of quitting. This move could save millions of lives; however, critical questions must be addressed before this policy can be

considered. First, what nicotine yield will reliably reduce cigarette use? Studies to date suggest that the optimal nicotine yield is below 0.2 mg, but precisely what yield reliably decreases smoking behavior in most smokers is still unknown. Second, would high tar, low nicotine cigarettes reduce toxicant exposure while maintaining the acceptability of these cigarettes?

This study will assess the behavioral, subjective and physiological effects of smoking cigarettes at the following approximate nicotine and tar yields: (1) 0.8 (±0.15) mg nicotine with 9 (±1.5) mg tars (typical nicotine and tar yields of commercially-available cigarettes; control condition); (2) 0.26 (±0.06) mg nicotine with 9 (±1.5) mg tar; (3) 0.12 (±0.03) mg nicotine with 9 (±1.5) mg tar; (4) 0.07 (±0.02) mg nicotine with 9 (±1.5) mg tar; (5) 0.03 (±0.01) mg nicotine with 9 (±1.5) mg tar; (6) 0.04 (±0.02) mg nicotine with 13 (±2) mg tar; (7) usual brand cigarettes. A range is provided because of the typical variation in tobacco products across batches. Overall, the results of this study will be used to evaluate the nicotine yield and tar level that reduces smoking, but also minimizes discomfort/dysfunction, dependence, biomarkers of exposure, and compensatory smoking. We will also assess compliance as an important indicator of acceptability. This study will also provide a detailed assessment of the mechanisms that may underlie the hypothesized change in behavior including: subjective effects of the cigarettes, perceived addiction/harm, abstinence-induced craving and withdrawal, and cigarette reinforcement.

Theoretical context:

Nicotine, like other drugs of abuse, is self-administered by a variety of animal species including humans (Corrigall & Coen, 1989; Goldberg et al., 1981; Henningfield & Goldberg, 1983; Rose & Corrigall, 1997), supporting the assertion that nicotine is a primary reinforcer capable of promoting behavior that results in its delivery. However, research has also indicated that nicotine is capable of conveying reinforcing properties on otherwise neutral stimuli (Caggiula et al., 2001; Goldberg et al., 1981; Palmatier et al., 2007). Indeed, cigarette smoke produces distinct and salient sensory cues that are frequently and consistently paired with nicotine delivery and are thought to be subject to a conditioned (Pavlovian) association with nicotine delivery (Rose & Levin, 1991). Hence, smoking may be maintained by both the primary reinforcing effects of nicotine and the subsequent conditioned effects of nicotine-associated stimuli (in addition to other pharmacologically active constituents). Consequently, the drastic reduction of nicotine would be expected to have two effects. First, smoking behavior promoted by the reinforcing properties of nicotine should decrease. Second, the conditioned effects of stimuli associated with nicotine should decrease over time as a consequence of extinction (i.e., repeated exposure to the conditioned stimulus in the absence of the unconditioned stimulus).

Effects of very low nicotine content (VLNC) cigarettes:

Studies of VLNC (e.g., 0.05 mg nicotine yield) cigarettes suggest that, acutely, they produce many effects in smokers that are qualitatively similar to normal nicotine content (NNC; e.g., 0.8 mg yield) cigarettes, but with somewhat reduced efficacy. VLNC cigarettes reinforce behavior (Shahan et al., 1999; Shahan et al., 2001), maintaining similar rates of self-administration as NNC cigarettes despite the fact that participants prefer NNC cigarettes when given a choice (Shahan et al., 1999). Compared to not smoking, VLNC cigarettes increase ratings of satisfaction and liking (Donny et al., 2007; Donny & Jones, 2009; Rose et al., 2000), although the magnitude of these effects is typically reduced compared to those produced by NNC cigarettes (Butschky et al., 1995; Gross et al., 1997; Robinson et al., 2000). VLNC cigarettes also reduce withdrawal and craving (Pickworth et al., 1999), although some symptoms (e.g., restlessness, impatience) may be more effectively alleviated by NNC cigarettes (Buchhalter et al., 2005). Much less is known

about the effects of VLNC cigarettes over an extended period of use. When only VLNC cigarettes were available in an inpatient setting, the number of cigarettes smoked and the motivation to smoke during periods of abstinence decreased over time (Donny et al., 2007). Reductions in reinforcement in the real world, however, may prove somewhat more difficult. Outpatient smoking rate remained unchanged for a week after switching to VLNC cigarettes (Benowitz et al., 2007; Benowitz et al., 2009; Donny & Jones, 2009), but declined significantly over a period of 3-6 weeks (Hatsukami et al., 2010). During this time, participants also reported minimal withdrawal symptoms and a reduction in nicotine dependence as measured by the FTND (Hatsukami et al., 2010). These data suggest that the conditioned reinforcing effects of cigarettes can be extinguished, but that the process is on the order of weeks rather than days. Finally, it is important to note that there is little evidence to suggest that prolonged use of VLNC cigarettes will result in a compensatory increase in smoking. Data available to date indicate that smoking is first maintained at a similar rate compared to preferred brand and then decreases over time (Donny & Jones, 2009; Hatsukami et al., 2010). Furthermore, participants tend to reduce the volume of smoke inhaled and demonstrate a decrease in expired CO (Donny & Jones, 2009; Hatsukami et al., 2010). These findings are in contrast to reports indicating an acute compensatory increase in total puff volume in participants smoking VLNC cigarettes (Strasser et al., 2007). Hence, VLNC cigarettes may produce a short-lived compensatory increase in smoking, but this effect likely dissipates quickly and is replaced by a decrease in smoke intake. Nevertheless, the study proposed below will closely monitor puff topography and markers of exposure to continue to address concerns about possible compensatory use of VLNC cigarettes.

In the context of considering a policy for low nicotine standards for cigarettes, the optimal upper limit for nicotine yields per cigarette is one that results in decreased abuse liability and exposure to nicotine and other constituents of tobacco smoke. The upper limit is expected to be less than, but likely near, the level of nicotine that results in sustained use and dependence. Benowitz and Henningfield (1994) proposed a value of approximately 0.2 mg (0.17 mg) of nicotine per cigarette as a threshold yield for establishing and sustaining addiction. More recent data support this estimate. Smoking rate for reduced nicotine content (RNC) cigarettes (i.e., above 0.2 mg but below NNC) tends to persist at the same or a somewhat higher rate than NNC cigarettes (Donny et al., 2007; Donny & Jones, 2009; Hatsukami et al., 2010). In contrast, much lower nicotine yields (e.g., 0.05 mg) result in reduced use and dependence (Donny et al., 2007; Hatsukami et al., 2010). Indeed, a 40% reduction in smoking behavior was observed over a 6-week period of smoking VLNC cigarettes with minimal experience of withdrawal symptoms, a reduction in FTND scores, and reduced exposure to the potentially harmful byproducts of tobacco (Hatsukami et al., 2010).

Despite the evidence that nicotine yield cigarettes below 0.2 mg will reduce cigarette use and exposure to smoke constituents, little data are available that have directly addressed the effect of different nicotine yields near or below this estimate. No published study has examined yields less than 0.05 mg. It is reasonable to expect that lower values could result in similar or more rapid declines in use and dependence, but possibly at the cost of increased withdrawal and reduced acceptability/compliance. At the upper end, three studies have evaluated cigarettes with yields >0.07 and ≤0.20 mg (Benowitz et al., 2007; Benowitz et al., 2009; Benowitz et al., 2012). In two of the studies, 20 participants smoked cigarettes with decreasing nicotine yield over the course of 6 weeks (1 week per yield). The third study was similar in design but nicotine yields were decreased on a monthly basis. The yields evaluated started in the usual brand range (0.8-0.9 mg) and decreased through several intermediate steps to 0.2 then 0.1 mg. Results

showed that although nicotine intake progressively declined, cigarette use remained stable throughout the assessment period (Benowitz et al., 2007; Benowitz et al., 2009, Benowitz et al., 2012). Given the relatively short duration of use, the lack of a decline in smoking is not surprising and consistent with other reports of even lower nicotine yield cigarettes (Donny & Jones, 2009; Hatsukami et al., 2010). Interestingly, one study observed a decrease in blood carboxyhemoglobin, total NNAL and polycyclic aromatic hydrocarbons at 0.1 mg (Benowitz et al., 2009) suggesting total smoke exposure was reduced, even if self-reported cigarettes per day were not. Furthermore, in both studies, nicotine dependence and subsequent use of preferred brand smoking decreased after the taper to 0.1 mg.

In sum, the available literature provides relatively little insight into the precise relationship between nicotine yield and potentially important outcomes in individuals smoking VLNC cigarettes over a prolonged period of time. The existing evidence supports the notion that nicotine yields <0.2 mg will likely produce the desired profile of effects, but additional information is needed

Cigarettes to be assessed in this study:
The cigarettes to be used in this study were made under an NIH contract with production being overseen by the Research Triangle Institute (referred to as "Spectrum cigarettes"). NIH currently has approximately 10 million of these cigarettes (of varying types) for research purposes. The cigarettes selected for the study span the range of yields likely to produce the hypothesized effects, as described above. The Spectrum cigarettes are not currently commercially available, although they are similar in many ways to marketed cigarettes (e.g., similar manufacturing, filter, paper, etc.).

The 13 mg tar yield condition was added to the design because it is similar to full flavor cigarettes. Some researchers believe it will make the VLNC cigarettes more acceptable because it will replace some of sensory properties (i.e., not taste too light or too much like air). Some studies have shown that high tar in cigarettes with normal nicotine content may increase harm (e.g., lung cancer risk) when smoked over a long period of time. However, whether the combination of very low nicotine and high tar yield results in increased tar exposure relative to preferred brand is unknown. We believe the cigarettes to be used here are unlikely to lead to adverse health effects in these participants because of the short period of exposure, the low chance of continued use of these high tar cigarettes because they are not commercially available, and, importantly, because of the very low nicotine content (which will likely lead to reduced smoking).

Screening Procedures

Recruitment:
Participants will be recruited through flyers, direct mailings, television, radio, newspaper, bus, and Craigslist advertisements that read "Smokers who want to try new cigarettes that may or may not lead to reduced smoking are wanted for research. Participants will be paid for participation." Those who call into the laboratory will be read a script briefly explaining the study. After verbal informed consent is received, the participants will be asked questions over the phone to determine initial eligibility. If eligible and interested, they will be scheduled for an in-person screening interview. 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 state issued photo ID to the screening visit. Acceptable forms of identification include a Driver's License, State Photo ID Card, Passport, or Military ID. If the potential participant does not have a valid state issued 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, potential participants will need to produce valid, state issued photo identification. The interviewer will confirm the age and identity of the participant. If the participant is not age 18 or older, he/she 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 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 all written measures and materials to the participant for the remainder of the screening visit. The interviewer will use a PowerPoint presentation to discuss the procedures, risks and benefits with the participant as well as his/her rights as a research participant. At the end of the presentation, the participant will be instructed to read several open-ended questions aloud and discuss the answers with the researcher. Only after the participant and the researcher are fully satisfied that the participant understands the purpose of the study, the confidentiality of the data, the procedures, the risks/benefits and his/her 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:

The following physiological measures will be collected, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

1) **Breath alcohol levels (BAL)** will be measured 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 received 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. 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 Smokerlyzer ED50 CO meter (Bedfont Instruments), a reliable and valid measure of recent smoking.
    a. **NicAlert Strips** will be used to asses urinary cotinine levels if a participant's carbon monoxide reading is less than or equal to 8 ppm.
4) **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 may

reschedule the interview but will need to be re-consented to ensure they have received adequate informed consent. They will be excluded if they are positive the second time.

5) **Pregnancy Tests (HCG detection)** will be performed for female participants with childbearing potential. We will also ask the date of last menstrual period and length of cycle.
6) **Blood pressure** and **heart rate** will be measured using a CritiCare monitor to help the licensed medical professional 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 at the end of the visit:

1) **Identifying Information Form** will include the participant's OnCore Subject Identifier, name, address (including the county of residence), email address, phone number, age, date of birth, and social security number.
    a. This form will be entered into the 'Identifying Information Access Database'.
        i. Each site will have a separate 'Identifying Information Access 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 Access Database (electronic version) separate from all other study data.
2) **Brief Medical History Questionnaire** to assess current diagnoses, symptoms and past health problems.
    a. Sections of the questionnaire will be entered into OnCore.
    b. The medications section will be transferred onto the 'Concomitant Medications' form and entered into OnCore.
3) **Prime MD**, a brief questionnaire developed for evaluation of mental disorders by primary care physicians (Spitzer et al., 1999).
    a. This questionnaire will be entered into Qualtrics.
4) **Beck Depression Inventory** (BDI; Beck, Ward, & Mendelson, 1961), 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 and then will be entered into the study databases by the interviewer at the end of the visit:

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** (Sheehan et al., 1997) 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 Qualtrics.
4) **Drug Use Questionnaire** (12 month and 1 month version)
    a. This questionnaire will be entered into Qualtrics.

5) **Smoking Cessation Therapy Use Questionnaire** to assess use of nicotine replacement therapy or smoking cessation medications to help participants quit smoking.
    a. This questionnaire will be entered into Qualtrics.

The following screening assessments will be 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 and 1 month version)
3) **Fagerström Test for Nicotine Dependence** (FTND; Heatherton et al., 1991)
4) **Smoking Stages of Change Algorithm** as well as a contemplation ladder to assess intention to quit smoking (DiClemente et al., 1991).

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Participants who make any response other than "not at all" on the suicidal ideation question of the Prime MD (Question 1i) or indicate suicidal ideation or attempt in the past month or a suicide attempt in the past 10 years on the 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 (BDI) and refer to the licensed on-site clinician for evaluation. In the event that no clinician is available, staff will ask the participant two questions to determine level of risk: "Are you feeling suicidal?" and "Do you have a plan to kill yourself today?" If the participant has a plan to kill himself/herself, staff will put the participant in contact with the National Suicide Prevention Lifeline at 1-800-273-8255. If the participant refuses to talk to the hotline and leaves, the study staff will call 911. They will also contact the Study Coordinator and Site PI to inform them of the situation as soon as possible. Additionally, they will contact the Project Coordinator (Rachel Denlinger) to inform her of the situation. If the participant does not have a plan to kill himself/herself, the study staff will recommend he/she speaks with the suicide hotline and inform him/her that he/she will not be eligible for the study. The participant will be paid $25 and provided with local mental health resources.

Additionally, any participant whose score on the Prime MD indicates Major Depressive Disorder will be administered the Beck Depression Inventory (BDI) on paper. The BDI will be submitted, along with the Prime MD, Brief Medical History Questionnaire, Brief Medical History Follow-up Questionnaire, and the MINI suicide subscale to the licensed medical professional for eligibility review. If he/she determines a participant with Major Depressive Disorder is eligible for study participation, the participant will complete the BDI on a weekly basis to monitor changes in his/her mood.

Inclusion Criteria:

1) Age 18+
2) Smoke an average of at least five cigarettes per day for at least 1 year
3) Breath CO levels > 8 ppm (if ≤ 8 ppm, then NicAlert Strip > 2)

Exclusion Criteria:

1) Intention to quit smoking in the next 30 days

2) Currently seeking treatment for smoking cessation
3) Currently using nicotine replacement therapies or other pharmacotherapies as cessation aid (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 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) Significant 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 in the past 3 months
10) Positive toxicology screen for any of the following drugs: cocaine, opiates, methadone, benzodiazepines, barbiturates, amphetamines, methamphetamines, and PCP
    a. Marijuana will be tested for but will not be an exclusionary criterion.
    b. Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines or methadone will not be excluded.
    c. Participants failing the toxicology screen will be allowed to re-screen once. These participants will need to be re-consented before being rescreened to ensure they have received adequate informed consent.
11) Breath alcohol level > 0.01
    a. Participants failing the breath alcohol screen will be allowed to re-screen once. These participants will need to be re-consented before being rescreened to ensure they have received adequate informed consent.
12) Binge drinking alcohol (more than 9 days in the past 30 days, 4/5 drinks in a 2 hour period (female/male))
13) Pregnant, trying to become pregnant or breastfeeding
14) Smoking 'roll your own cigarettes' exclusively
15) Currently taking anticonvulsant medication
16) CO reading >80 ppm
17) Systolic BP greater than or equal to 160
    a. Participants failing for blood pressure will be allowed to re-screen once.
18) Diastolic BP greater than or equal to 100
    a. Participants failing for blood pressure will be allowed to re-screen once.
19) Systolic BP below 90 and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
    a. Participants failing for blood pressure will be allowed to re-screen once.
20) Diastolic BP below 50 and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
    a. Participants failing for blood pressure will be allowed to re-screen once.
21) Heart rate greater than or equal to 115 bpm
    a. Participants failing for heart rate will be allowed to re-screen once.
22) Heart rate lower than 45 bpm and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint).
    a. Participants failing for heart rate will be allowed to re-screen once.
23) Indicating any suicidal ideation in the past month or suicide attempts in the past 10 years.

24) Inability to independently read and comprehend the consent form and other written study materials and measures.
25) Having participated in a research study during the past three months in which the participant:
    a. Smoked a cigarette that was not his/her usual brand cigarette for more than one day
    b. Used any tobacco products beyond normal use for more than one day
    c. Used any nicotine replacement products or smoking cessation medications for more than one day

Children under age 18 are excluded because they cannot legally buy cigarettes. Those with unstable medical, psychiatric, or medication conditions (condition and/or medication changes in the past 3 months) 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, 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. 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. We will exclude pregnant and nursing women and anyone with current or recent alcohol or drug abuse problems 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 10 years will be excluded from the study for safety concerns. Individuals who smoke 'roll your own' cigarettes exclusively will be excluded from the study because we will be unable to standardize their baseline smoking behavior. Participants that are currently prescribed anticonvulsant medication will be excluded because this medication can interfere with the biomarkers. If an individual has recently participated in a smoking research study that changed his/her smoking behavior this person would be excluded because he/she would not have a stable smoking baseline. Because participants are required to complete portions of the protocol independently both in the lab and at home, they will need to be able to independently read and comprehend the study materials.

Eligibility Determination:
The final eligibility of the participant will be determined by a licensed medical professional (MD, DO, NP, PA or CRN) 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 licensed medical professional for review as well. Additionally, if the participant's score on the Prime MD indicates Major Depressive Disorder, then the Prime MD along with the Beck Depression Inventory will be submitted 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 licensed 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 prior to the first baseline visit. The licensed 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 he/she is taking, then he/she will not be automatically excluded. The interviewer will record the positive result into OnCore and perform an eligibility override to prevent the system from marking the participant as ineligible. The interviewer will make note of this when he/she submits the forms to the licensed medical professional for final eligibility determination.

Once all the screening procedures have been completed, researchers will pay participants $25 for their time as long as they pass the drug and breath alcohol tests and meet the minimum requirements for carbon monoxide or NicAlert levels. Those participants who do not pass these tests or meet these requirements will be dismissed from the study without payment. Marijuana will be tested for but will not be an exclusionary criterion. If a participant does not pass the drug test but has a current, valid prescription that would explain the failed test he/she will not be automatically excluded and will still receive $25. Participants who meet all other eligibility criteria, sans the medical criteria, will be scheduled for the first baseline visit.

At the end of the screening session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, 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, 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 significant medical problems including:
    a. Cardiovascular Diseases: Coronary heart disease, heart attack, stroke, peripheral vascular disease, reduced blood circulation, abdominal aortic aneurysm
    b. Respiratory Diseases: Emphysema, bronchitis, and chronic airway obstruction
    c. Cancers: Cancer of the lung, bladder, cervix, esophagus, kidney, larynx, mouth, pancreas, throat, and stomach; leukemia
    d. Other Health Risks Associated with Smoking: Including but not limited to infertility, lower bone density in postmenopausal women, and hip fracture in women
    e. Death

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. Due to the altered nicotine levels, there could be a change in their cigarette use including the manner in which they inhale the smoke. Smoking the study cigarettes does not provide any less risk than their usual brand cigarette and could pose increased health risks. Participants may also experience increases in levels of carbon monoxide, a gas from smoke.

7) Smoking Withdrawal: Participants may experience smoking withdrawal symptoms during this study. The symptoms can be uncomfortable but are typically of minimal risk. 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 cigarette at the end of the study they may experience mild and transient nausea, dizziness, and lightheadedness.

9) Risk to Fetus**:** Smoking during pregnancy can lead to 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 cigarettes consumption could adversely affect psychiatric conditions

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 screening visit, before randomization during the Baseline 2 visit and at the Week 6 visit. If a participant becomes pregnant during the study, she will be withdrawn from the study. Approximately 30 days after being withdrawn or having a positive pregnancy test at the Week 6 visit, the research staff will call 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

Following baseline, participants will be randomized into one of seven cigarette conditions. Participants in each condition will be assigned a cigarette that matches their menthol preference.

Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
1	NRC600	CN	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
1	NRC601	CN-Men	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
2	NRC400	RN	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
2	NRC401	RN-Men	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
3	NRC300	RN	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
3	NRC301	RN-Men	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
4	NRC200	RN	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
4	NRC201	RN-Men	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
5	NRC102	RN	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
5	NRC103	RN-Men	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
6	NRC104	RN-HT	0.04 ± 0.02	13 ± 2	0.02 - 0.06
6	NRC105	RN-HT-Men	0.04 ± 0.02	13 ± 2	0.02 - 0.06

Non-Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
7	N/A	USUAL BRAND	N/A	N/A	N/A
7	N/A	USUAL BRAND - Men	N/A	N/A	N/A

*Legend:	
RN-HV	Reduced Nicotine-High Ventilation
RN-HV-Men	Reduced Nicotine-High Ventilation Menthol

RN	Reduced Nicotine
RN-Men	Reduced Nicotine-Menthol
RN-HT	Reduced Nicotine-High Tar
RN-HT-Men	Reduced Nicotine-High Tar-Menthol
CN	Conventional Nicotine
CN-Men	Conventional Nicotine-Menthol
CN-HT-Men	Conventional Nicotine-High Tar-Menthol
LTNR	Low Tar/Nicotine Ratio
LTNR-Men	Low Tar/Nicotine Ratio-Menthol

During the experimental period, participants will be provided with a 14-day supply of research cigarettes or their usual brand cigarettes. 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. Participants will be instructed to use the research cigarettes for 6 weeks, at which point they are to discontinue product use.

They will be asked to refrain from use of other non-study cigarettes during the study period. However, they will be told there is not a penalty for use of non-study cigarettes, and that it is crucial for them to report any use of non-study cigarettes or other nicotine or tobacco products. Throughout the baseline and experimental periods, an Interactive Voice Response (IVR) system will be used on a daily basis to record the number of study cigarettes and non-study cigarettes used the previous day. During the baseline and first experimental week, participants will also answer daily IVR questions about their mood. Participants will be seen weekly for assessments. Brief standardized review sessions focusing on compliance with the study cigarettes and other study procedures will be provided at each visit. At the end of the 6-week trial, participants will undergo an assessment of withdrawal, craving, and cognitive function following a brief period of abstinence.

Baseline Period:
This study will use a one 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, reduce reactivity to the daily cigarette monitoring, and reduce participant burden. During the two baseline sessions, participants will complete subjective questionnaires, assessments of cognitive functioning, and smoking topography. Each visit will last approximately two hours or less. At the end of each baseline session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, and to assess the truthfulness of the participant in regards to self-report of tobacco use.

Visit scheduling requirements for baseline period:
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/she will need to be re-screened. This will be entered into OnCore as an 'Unscheduled Visit'. The participant will need

to be re-consented but will maintain the original OnCore Subject Identifier. The ideal target window separating Baseline 1 and Baseline 2 is between 6 and 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 he/she will not be rescheduled and will be discontinued from the study.

Physiological measures collected at Baseline 1, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

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

Physiological measures collected at Baseline 2, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

1) BAL
2) Weight
3) Three CO readings (initial visit CO, pre-topography and post-topography)
4) Blood Pressure
5) Heart Rate
6) Urine Pregnancy Test, if applicable

The following assessments will be administered as an interview and then entered into the study databases by the interviewer at the end of the visit:

1) **Concomitant Medications Form**
    a. This form will be entered into OnCore.
2) **Medical Event Form**, if applicable, will assess the nature, severity, duration, action taken, and outcome of medical event.
    a. This form will be entered into OnCore.
3) **Health Changes Questionnaire** which will assess any weekly health changes.
    a. This assessment will be entered into Qualtrics.
4) **Timeline Follow Back Questionnaire,** which will assess other tobacco and nicotine product use as well as alcohol and marijuana use during the past 14 days. (Baseline 2 only)
    a. This assessment will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source documents only:

1) **BDI**, if applicable

The following assessments will be administered using Qualtrics in order to standardize assessments across sites and projects:

<u>Baseline 1 Only</u>:

1) **Wisconsin Index of Smoking Dependence Motives-Brief** (WISDM; Piper et al., 2008), a measure of tobacco dependence
2) **Perceived Health Risks Rating** (Hatsukami et al., 2010), a measure of the perceived addictive potential and other health risks associated with cigarettes
3) **Perceived Stress Scale - 4 item** (PSS-4; Cohen, Kamarck, & Mermelstein, 1983), which measures the degree to which life situations are appraised as stressful

4) **Positive and Negative Affect Schedule** (PANAS; Watson, Clark, & Tellegan, 1988), which measures symptoms of positive and negative affect.
5) **Center for Epidemiological Studies-Depression Scale** (CES-D; Radloff, 1977), which measures symptoms of depression
6) **Short Michigan Alcohol Screening Test** (SMAST; Selzer et al., 1975), which measures past alcohol use
7) **Drug Abuse Screening Test Brief Version** (DAST-10; Gavin, Ross, & Skinner, 1989), which measures prior drug use and abuse

Baseline 1 & 2:

1) **Respiratory Health Questionnaire**, a measure of cough, shortness of breath and other respiratory symptoms
2) **Minnesota Nicotine Withdrawal Scale** (MNWS; Hughes & Hatsukami, 1986), a measure of nicotine withdrawal
3) **Questionnaire of Smoking Urges-brief scale - Usual Cigarette** (QSU; Cox, Tiffany, & Christen, 2001; Tiffany & Drobes, 1991), which measures the urge to smoke
4) **Cigarette Evaluation Scale** (CES; Westman, Levin, & Rose, 1992), which measures responses to cigarettes (e.g., reward, satisfaction)

Baseline 2 Only:

1) **Environmental and Social Influences on Tobacco Use Questionnaire** (adapted from Nondahl, Cruickshanks, & Schubert, 2005), which measures tobacco smoke exposure at home, work and socially
2) **Cigarette Purchase Task – Usual Brand Version** (Jacobs & Bickel, 1999; MacKillop et al., 2008) 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 subject reports zero consumption) and Pmax (the price at which Omax occurs). This task will indicate whether prolonged VLNC cigarette 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.

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Interactive Voice Response System:

Participants will also 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 as well as withdrawal symptoms the week before and after randomization. Participants will be provided a study cell phone if they have unreliable telephone access, do not have enough monthly cell phone minutes or prefer not to use their own phone.

Baseline 1 biological specimens collected, stored, and entered into caTissue:

1) **Saliva sample for DNA analysis:**

Participants will be asked to provide a saliva sample for DNA analysis. The sample will be collected using an OraGene kit. Saliva samples will be sent quarterly to be stored at the University of Minnesota. The DNA sample will be used to determine if there are links between genes and levels of tobacco constituents, behavior, mood, brain functioning, the harmful effects of smoking. Participants who chose not to provide a DNA sample will still be allowed to participate in the study.

Baseline 2 biological specimens collected, stored, and entered into caTissue:

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 stored at temperatures no more than -20°C. Urine samples will be sent quarterly to be analyzed and stored at the University of Minnesota. The tobacco-specific carcinogen biomarkers are total NNAL and total NNN. We will also assess total nicotine equivalents and minor alkaloids, including anatabine, anabasine, myosmine, and nornicotine. Platelet activation will be assessed with the cardiovascular biomarker 11-dehydroTXB2. If a participant forgets to bring his/her urine sample, then an on the spot urine sample will be collected.

2) **Saliva sample for cotinine assessment**:
Participants will be asked to provide two saliva samples during the second baseline session for assessment of nicotine metabolite ratio (NMR), an indicator of CYP2A6 enzyme activity. Participants must wait 30 minutes after arrival to the lab before collecting the first saliva sample. During this time participants cannot eat, drink, chew gum or smoke cigarettes. After collecting the sample, provide time for the participant to eat and/or drink before waiting another 30 minutes before collecting the second saliva sample. The second saliva sample must be collected prior to puff topography. Samples will be stored at temperatures no more than -20°C. Saliva samples will be sent quarterly to be analyzed and stored at the University of Minnesota.

Baseline 2 biological specimens collected, stored, and shipped to the CDC:

1) **Cigarette butt from puff topography:**
The cigarette butt will be collected in a freezer vial. 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.

2) **24-hour cigarette butt collection:**
Cigarette butts will be collected during the 24 hours prior to the biosamples first void urine collection. Participants will be provided with 20 tins and a collection box. They will be instructed to record the time each cigarette was smoked and put the cigarette butts into the collection box in the order they were smoked. Both usual brand and non-usual brand 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.

Cognitive Tasks:
Cognitive functioning will be assessed using a battery of computer-based assessments. We will assess domains that are theoretically linked to smoking and likely to be sensitive to nicotine

abstinence (Heishman, 1999; Kleykamp et al., 2005; Rycroft et al., 2006). Prior to test administration, participants will be trained to ensure their understanding of each test. Tests will be administered on a desktop computer.

1) **N-Back (0,2) Task** (Ernst et al., 2001): A measure of working memory in which participants view serially presented letters on a computer. They must indicate whether each letter presented is the same or different from the letter presented a specified number of positions back in the string of letters (e.g. 2-back).
2) **2-Letter Search** (Ernst et al., 2001): A measure of focused attention in which participants view strings of letters on a computer screen looking for whether each string contains or does not contain two target letters.
3) **Continuous Performance Test** (CPT; Myers et al., 2008): A measure of sustained attention, participants must monitor a string of stimuli (e.g. letters) serially presented on a computer screen monitoring for presentation of a target stimulus. The task is balanced so that they either must respond, or inhibit a response each time the target is presented.
4) **Heishman Arithmetic Test** (Myers et al., 2008): A measure of information processing speed and basic arithmetic. Participants view solved single digit addition or subtraction equations presented on a computer screen and must indicate, as quickly as possible, whether the solution provided is correct or incorrect.

Puff Topography:

Puff Topography, a precise measure of smoking behavior (Brauer et al., 1996; Herning et al., 1981; Robinson & Forbes, 1975), will be used to examine whether prolonged use of the experimental cigarettes affects topography measures that may indicate smoking compensation (Strasser et al., 2007). Puff topography will be assessed using a handheld topography device that provides a valid measurement of puff number, puff volume, inter-puff interval and other indices (Blank et al., 2009). Carbon monoxide readings will be collected before and 15 minutes after puff topography. The cigarette butt will be collected in a freezer vial, stored at room temperature, and sent to the CDC for solanesol analysis.

Randomization:

At the end of the second baseline visit, participants will be randomized to one of seven experimental conditions and will receive a 14-day supply of research cigarettes or usual brand cigarettes. This will ensure adequate availability of cigarettes in the numerous locations participants typically keep a supply (home, work, vehicle, etc.) as well as avoid expending the entire supply if they miss a scheduled visit.

The Administrative Core for the Center for the Evaluation of Nicotine in Cigarettes (CENIC) will be responsible for removing all identifying information from cigarettes received from the Research Triangle Institute (RTI), labeling each carton with a blind code, assigning product using this blind code based on the randomization schedule being provided by the CENIC Biostatistics Core, and shipping cigarettes to each site as needed based on recruitment. Each site will be responsible for tracking product received and distributed to participants, collecting unused product from participants, and destroying unused open packs. The participants, investigators and study staff will not have knowledge of which product is given to a participant or whether different participants received the same or different product.

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 laboratory each week. Research staff will complete 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. Unused cigarettes will be re-distributed to the participants during Weeks 1-5. During Week 6, any remaining unused cigarettes returned by the participants will be collected by the research staff.

If participants lose more than two packs of cigarettes and require an unscheduled visit to the laboratory to supplement their supply, they will be told the next time they lose more than two packs they will have to wait until their next scheduled appointment to receive more cigarettes.

Experimental Period:
Participants will be seen weekly throughout the 6-week experimental period. Weeks 2 and 6 will take approximately 1-2 hours each. All other sessions will last 1 hour or less. At the end of each experimental session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, and to assess the truthfulness of the participant in regards to self-report of tobacco use and compliance to study procedures.

Visit scheduling requirements for experimental period:
OnCore will automatically generate the ideal visit calendar once the participant has been put 'On Study'. The perfect scheduling window between each visit is 7 days based on the date of the Baseline 2 Visit. For additional scheduling requirements, refer to the '*Scheduling Visits SOP*'. If a participant misses a visit and is not able to reschedule during the window, that visit will not be 'made-up' in the future. All measures that were not completed will be considered missing data and will not be collected during future visits. If a visit mistakenly occurs outside of the designated window, this is a protocol deviation. A 'Non-Medical Event Form' will need to be completed and tracked in OnCore.

If a participant is not able to attend his/her Week 6 visit, then it should be rescheduled even if it is outside of the scheduling window. This will be documented as a protocol deviation.

Experimental Sessions 1, 3, 4, and 5 Procedures:

Physiological Measures Collected, recorded on paper, and entered into OnCore by interviewer at the end of 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 the study databases by the interviewer at the end of the visit:

1) Concomitant Medications

    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow Back Questionnaire
    a. Will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source document only:

1) BDI, if applicable

The following assessments will be administered using Qualtrics:

1) Respiratory Health Questionnaire
2) MNWS
3) QSU brief - Usual Brand Cigarette
4) QSU brief– Study Cigarette
5) Cigarette Evaluation Scale

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Experimental Sessions 2 and 6 Procedures:

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

1) BAL
2) Weight
3) Three CO readings (initial visit CO, pre-topography and post-topography)
4) Blood Pressure
5) Heart Rate
6) Urine sample for drug test and pregnancy test (if applicable) (Week 6 only)

Biological specimens collected, stored, and entered into caTissue:

1) First void urine sample

Biological specimens collected, stored and shipped to the CDC:

1) Cigarette butt from puff topography
2) 24-hour cigarette butt collection

Urine sample for smoking biomarker assessment:

Participants will be asked to bring a urine sample (first void of the day) to the week 2 and 6 sessions for biomarker assessment. Samples will be stored at temperatures no more than -20°C. Urine samples will be sent quarterly to be analyzed and stored at the University of Minnesota. The tobacco-specific carcinogen biomarkers are total NNAL and total NNN. We will also assess total nicotine equivalents and minor alkaloids, including anatabine, anabasine, myosmine, and nornicotine. Platelet activation will be assessed with the cardiovascular biomarker 11-dehydroTXB2. If a participant forgets to bring his/her urine sample, then an on the spot urine sample will be collected.

Cigarette butt from puff topography:
The cigarette butt will be collected in a freezer vial. 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.

24-hour cigarette butt collection:
Cigarette butts will be collected during the 24 hours prior to the biosamples first void urine collection. Participants will be provided with 20 tins and a collection box. They will be instructed to record the time each cigarette was smoked and 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.

The following assessments will be administered as an interview and will be entered into the study databases by the interviewer at the end of the visit:

1) Concomitant Medications
    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow Back Questionnaire
    a. Will be entered into Qualtrics.
5) Drug Use Questionnaire - 1 month version (week 6 only)
    a. Will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source documents only:

1) BDI, if applicable

The following assessments will be administered using Qualtrics:

1) Respiratory Health Questionnaire
2) MNWS
3) QSU brief - Usual Cigarette
4) QSU brief – Study Cigarette
5) FTND
6) Cigarette Evaluation Scale
7) Perceived Health Risks Questionnaire
8) Smoking Stages of Change Algorithm and Contemplation Ladder
9) Cigarette Purchase Task - Usual Brand Cigarette Version
10) Cigarette Purchase Task – Study Cigarette Version
11) PANAS
12) Perceived Stress Scale – 4 item
13) Alcohol Use Questionnaire -1 month version (week 6 only)
14) Environmental and Social Influences on Tobacco Use Questionnaire (week 6 only)
15) CESD (week 6 only)
16) WISDM-Brief (Week 6 only)

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Participants will also complete the following tasks:

1) Cognitive tasks
2) Puff Topography

Interactive Voice Response System:

Participants will continue to use the IVR system on a daily basis throughout the experimental period to record the number of study cigarettes smoked per day and use of non-study cigarettes. During the first week after randomization, the IVR system will collect information about withdrawal symptoms.

Product and Procedures Compliance Review Sessions:

At each visit, Baseline 2 through Week 5, participants will be counseled about their use of the study cigarettes. Participants will be asked about any concerns or obstacles associated with use of the study cigarettes. The importance of honest self-reporting will be stressed. Participants will be told that they will not be penalized for use of other nicotine or tobacco products and 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. Additionally, participants will be counseled about their IVR completion, visit attendance, task engagement and product accountability. Refer to the '*Product and Procedures Compliance Review Sessions SOP*' for more information.

Quit Attempts During the Study Protocol:

At each weekly session, we will ask the participant if he/she is currently abstaining from smoking with the intention of quitting. If the answer is no, then we will also ask if he/she is planning to quit smoking prior to his/her next scheduled visit.

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 visits, but no puff topography
- 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 choses 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 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 that 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 as to avoid unwanted cues to smoke.

Abstinence Assessment Session:
After the week 6 visit, participants will be required to come back for one additional visit the following day. During this visit, participants will have been encouraged to abstain from smoking until their next scheduled visit (approximately 24 hours later). The abstinence assessment session should be scheduled no less than 18 hours and no more than 30 hours after the Week 6 visit. Abstinence will be verified by expired breath carbon monoxide levels that have decreased by at least 50% from the measure taken during the Week 6 visit (post-topography CO) or less than 6 ppm. This session will allow us to determine whether the experimental cigarettes have reduced the effects of abstinence on these measures relative to the control conditions. If the participant does NOT meet abstinence criteria, he/she will not receive the $90 for the visit.

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

1) BAL
2) CO
3) Blood Pressure
4) Heart Rate

The following assessments will be administered as an interview and will be entered into study databases by the interviewer at the end of the visit:

1) Concomitant Medications
    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow-Back
    a. Will be entered into Qualtrics.

The following assessments will be administered using Qualtrics:

1) MNWS
2) QSU-brief - Usual Cigarette
3) QSU-brief - Experimental Cigarette
4) Cigarette Purchase Task - Usual Brand Cigarette Version
5) Cigarette Purchase Task - Study Cigarette Version

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Participants will also complete the following task:

1) Cognitive tasks

Participants who do **NOT** meet abstinence criteria will be required to complete the following assessments:

1) BAL
2) CO
3) Blood Pressure
4) Heart Rate
5) Concomitant Medications
6) Health Changes Questionnaire
7) Medical Event Form, if applicable
8) Timeline Follow-Back

Participant Compensation:
Participants will receive $25 for completing the screening visit, regardless of enrollment as long as the participant passes the drug test, breath alcohol test, and meets the minimum requirements for carbon monoxide or NicAlert levels. Participants who do not pass these tests will be dismissed from the screening visit without payment, except in the event they can produce a prescription for the medication that caused them to fail the drug test.

- Screening Session: $25
- Short sessions: $25 per session ($125 total)
- Long sessions: $75 per session ($225 total)
- Abstinence Session: $90 (meets abstinence criteria of less than 6 ppm and/or a 50% reduction from Week 6 CO)
- Completion and on time bonus: $200 (attends all 10 visits)
- IVR compliance: up to $160
- 30 day follow up phone call: $10
  Total: $835

Participants are free to discontinue at any time and will receive compensation for the sessions completed at the same rate listed above. However, they will not receive the completion bonus.

End of Study:
After a participant has completed all study procedures and has been paid for participation the research assistant should read the following script and give the participant the *Clearing the Air Manual.*

"Before you go, we want to encourage you to try to continue to be abstinent as long as possible. Although the study is over and abstinence is not required, you may find it easier to quit as a result of your participation. We would like to provide you with some resources should you decide to try to abstain from smoking (give "Clearing the Air" and hotline information). Please also feel free to consult with your physician and use any medications he/she deems appropriate. We will call you in approximately 30 days to ask about your smoking since leaving the study. There is no right answer and we know how difficult quitting can be. Please just answer honestly. The call will take less than 5 minutes, and we will compensation you for your time by giving you another $10. Thanks again for your participation."

The following assessments will be administered using Qualtrics:

1) End of Study Questionnaire

30 Day Follow up Phone Call:
Participants will receive a follow-up phone call between 25 and 35 days after the abstinence assessment session to assess their smoking patterns. The phone questionnaire will last less than five minutes. The questionnaire will ask if the participant is still smoking, how much and whether he/she has attempted to quit smoking since the end of the study. Additionally, any Medical Event Forms that remain open from the last session will be discussed. If the participant became pregnant during the study, this would have been recorded as a medical event. During this phone call, the research assistant will confirm her due date. This event will remain open until delivery. At that time the licensed medical professional will contact the participant to ask a few questions about the baby's health and will update the Medical Event Form.

Once a participant has completed all study procedures and all open events have been closed, the PI will review the participant's binder and sign a form indicating study completion for that participant.

Study Debriefing:
After data collection is complete, participants will receive 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 Bioinformatics Core for at least 7 years after study completion.

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.
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 participant indicates she is pregnant or has a positive pregnancy test at Week 2, she will be withdrawn from the study, and this event will remain open until delivery. At that time the licensed medical professional will contact the participant to ask a few questions about the baby's health and will update the open 'Medical Event Form'. A positive pregnancy test at Week 6 will trigger a 'Medical Event Form' to be completed but will not result in withdrawal since she is no longer receiving study product.

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 PI or Licensed Medical Professional:

1) Cigarettes per day increase: Continued participation will be evaluated by the site PI 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 115 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 115 bpm for heart rate, a 'Blood Pressure and Heart Rate Symptom Checklist' and 'Medical Event Form' 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, a 'Medical 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 'Medical 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 PI 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 PI can withdraw him/her from the study at the PI's discretion.

6) If a participant fails to attend his/her Baseline 2 Visit (Randomization) within the 21 day allowable visit window, he/she will not be eligible to reschedule this visit or continue participation in the study.

Investigational Tobacco Product

The Co-Directors of this Center grant, Drs. Donny and Hatsukami, have received an Investigational Tobacco Product (ITP) application to the FDA to cover the experimental cigarettes being used in this study. This application encompasses all Project 1 sites.

Certificate of Confidentiality

To help protect the participant's privacy, the Co-Directors of Center grant, Drs. Donny and Hatsukami, have received a Certificate of Confidentiality from the National Institutes of Health. With this certificate, the researchers cannot be forced to disclose 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 themself 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 Outcome Variables:

- Number of cigarettes smoked per day
  - Study cigarettes
  - Combined study and non-study cigarettes

Secondary Outcome Variables:

- Measures of compliance: non-study cigarette use, drop-out rate
- Measures of discomfort/dysfunction: MNWS, PANAS, QSU, PSS-4, CESD

- Measures of other health-related behaviors: breath alcohol, urine drug screen, TLFB-marijuana use, Alcohol Use Questionnaire, Drug Use Questionnaire, DAST, SMAST, weight
- Measures of nicotine/tobacco dependence: FTND, WISDM
- Measures of tobacco exposure: CO, total nicotine equivalents, NNN, NNAL, minor alkaloids
- Measures of smoking context: Environmental and Social Influences on Tobacco Use
- Measures of intention to quit: Stages of Change, Contemplation Ladder
- Measures of compensatory smoking: puff topography, filter analysis
- Measures of other tobacco use: TLFB-other tobacco
- Measures of cigarette characteristics: CES, Cigarette Purchase Task
- Measures of cognitive function: N-back, 2-Letter Search, CPT, Arithmetic Task
- Measures of cardiovascular function: heart rate, blood pressure, urine 11-dehydroTXB2
- Measures of perceived risk: Perceived Health Risk Questionnaire

Statistical Approach

The primary objective of this trial is to evaluate the effects of cigarettes varying in nicotine content. Our general approach to statistical analysis will be as follows. First, we will compare the four primary experimental conditions, conditions (2) – (5), to the primary control condition, condition (1), for the number of cigarettes smoked per day at six weeks. Comparisons with conditions (6) and (7) will provide further insight into the impact of tar yield and product switching.

(1) Baseline characteristics including demographics and smoking history will be compared between the treatment groups to identify any baseline imbalances after randomization. Discrete variables will be summarized by frequencies and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate. Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA.

(2) Continuous outcomes will be summarized by the mean, standard deviation, median and range, while categorical outcomes will be summarized by frequencies and percentages. Skewed continuous outcomes will be log-transformed or square root-transformed as appropriate; for example we conventionally analyze cotinine on log scale.

(3) We expect groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis for all endpoints will only adjust for the baseline value of that endpoint (for precision). However, a secondary analysis will be completed adjusting for age, sex and race, along with any other covariates that differ across treatment groups at baseline with a p-value less than 0.20.

(4) All analyses will be completed using SAS (version 9.2 or 9.3) or R. P-values less than 0.05 will be considered statistically significant with the exception of the analysis of our primary analysis, where p-values less than 0.0125 will be considered significant after a Bonferroni multiple comparisons adjustment.

Our primary endpoint, cigarettes per day at 6 weeks, will be summarized by treatment group and analyzed using linear regression adjusting for cigarettes per day at baseline. We will first compare the primary experimental conditions, conditions (2) – (5), to the primary control condition, condition (1). P-values less than 0.0125 will be considered significant. A pairwise comparison will be completed among experimental conditions that are significantly different than from the control condition using an appropriate Bonferroni adjustment. A secondary analysis of the primary endpoint will be completed adjusting for age, sex and race, along with any other covariates that differ across treatment groups at baseline with a p-value less than 0.20. Finally, cigarettes per day will be analyzed using a linear mixed model to evaluate trends in the difference in the number of cigarettes per day over time.

Secondary endpoints will be analyzed following the same approach as the primary endpoint. The primary analysis of our secondary endpoints will use linear regression and adjust only for the baseline value. Secondary analyses will consist of an adjusted analysis and a repeated measures analysis using a linear mixed model. In addition, we will also complete pre-planned subgroup analysis by sex (men vs. women), race (white vs. black) and menthol status (non-menthol versus menthol).

Power Analysis

Power analyses are based on two-sided, two-sample t-tests at the 1.25% level to control for multiple comparisons of VLNC cigarettes to NNC controls (4 nicotine yields; 4x1.25 = 5% level). With 96 completers per group (based on 20% attrition over the 6 week period), we will achieve 80% (90%) power to detect an effect size of 0.486 (0.550) which is less than the effect of VLNC vs. NNC cigarettes observed by Hatsukami et al. (2010) for cigarettes per day (CPD; 0.991), cotinine (2.096), FTND (0.603), and withdrawal (0.512). Given equal recruitment of males and females, we will also be able to detect an effect size of 0.693 (0.785) for analyses focused on a single sex (n=48/group).

OnCore Subject Identifier

The subject identifier is an alpha-numeric combination. Example: A001 would be University of Pittsburgh's first subject.

Site Identifier:
A = University of Pittsburgh
B = University of Minnesota Masonic Cancer Center
C = Brown University
D = Johns Hopkins University
E = University of Pennsylvania
F = Duke University
G = University of Texas MD Anderson Cancer Center
H = University of California, San Francisco
I = University of Minnesota Medical School Duluth
J = University of South Florida Moffitt Cancer Center

Subject ID:
001-899

OnCore 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
05= Week 5 clinic visit
06= Week 6 clinic visit
07= Week 6 + day 1 lab session (Abstinence Session)
30= 30 day follow-up phone call
99= UNSCHEDULED/ADDITIONAL visit

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PROJECT 1, STUDY 1:

INVESTIGATING THE IMPACT OF NICOTINE USING SPECTRUM CIGARETTES

STUDY PROTOCOL

Table of Contents

Abbreviations

- VLNC: Very low nicotine content
- RNC: Reduced nicotine content
- NNC: Normal nicotine content
- CPD: Cigarettes per day
- CO: Carbon monoxide
- BAL: Breath alcohol levels
- BP: Blood pressure
- HR: Heart rate
- BPM: Beats per minute
- NMR: Nicotine metabolite ratio
- NNN: *N*'-nitrosonornicotine
- NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
- BDI: Beck's Depression Inventory
- FTND: Fagerström Test for Nicotine Dependence
- WISDM: Wisconsin Index of Smoking Dependence Motives
- CESD: Center for Epidemiological Studies Depression Scale
- TLFB: Timeline Follow Back
- MNWS: Minnesota Nicotine Withdrawal Scale
- PANAS: Positive and Negative Affect Scale
- QSU: Questionnaire of Smoking Urges
- CES: Cigarette Evaluation Scale
- PSS: Perceived Stress Scale
- SMAST: Short Michigan Alcohol Screening Test
- DAST: Drug Abuse Screening Test
- CPT: Continuous Performance Task
- IVR: Interactive Voice Response

CENIC Project 1, Study 1 Protocol

Objective:
Project 1, Study 1 will evaluate the relationship between nicotine yield of very low nicotine content cigarettes and cigarettes smoked per day, nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. We will also consider differences between conditions in compliance with product use.

Background Information:
About 5 million people die every year world-wide because of tobacco. The Family Smoking Prevention and Tobacco Control Act (FSPTCA; Congress, 2009) gives the FDA the authority to limit (but not eliminate) the nicotine content of cigarettes. Drastically reducing nicotine content has the potential to reduce cigarette reinforcement and dependence, which in turn may result in less consumption per individual smoker and a greater likelihood of quitting. This move could save millions of lives; however, critical questions must be addressed before this policy can be considered. First, what nicotine yield will reliably reduce cigarette use? Studies to date suggest that the optimal nicotine yield is below 0.2 mg, but precisely what yield reliably decreases smoking behavior in most smokers is still unknown. Second, would high tar, low nicotine cigarettes reduce toxicant exposure while maintaining the acceptability of these cigarettes?

This study will assess the behavioral, subjective and physiological effects of smoking cigarettes at the following approximate nicotine and tar yields: (1) 0.8 (±0.15) mg nicotine with 9 (±1.5) mg tars (typical nicotine and tar yields of commercially-available cigarettes; control condition); (2) 0.26 (±0.06) mg nicotine with 9 (±1.5) mg tar; (3) 0.12 (±0.03) mg nicotine with 9 (±1.5) mg tar; (4) 0.07 (±0.02) mg nicotine with 9 (±1.5) mg tar; (5) 0.03 (±0.01) mg nicotine with 9 (±1.5) mg tar; (6) 0.04 (±0.02) mg nicotine with 13 (±2) mg tar; (7) usual brand cigarettes. A range is provided because of the typical variation in tobacco products across batches. Overall, the results of this study will be used to evaluate the nicotine yield and tar level that reduces smoking, but also minimizes discomfort/dysfunction, dependence, biomarkers of exposure, and compensatory smoking. We will also assess compliance as an important indicator of acceptability. This study will also provide a detailed assessment of the mechanisms that may underlie the hypothesized change in behavior including: subjective effects of the cigarettes, perceived addiction/harm, abstinence-induced craving and withdrawal, and cigarette reinforcement.

Theoretical context:
Nicotine, like other drugs of abuse, is self-administered by a variety of animal species including humans (Corrigall & Coen, 1989; Goldberg et al., 1981; Henningfield & Goldberg, 1983; Rose & Corrigall, 1997), supporting the assertion that nicotine is a primary reinforcer capable of promoting behavior that results in its delivery. However, research has also indicated that nicotine is capable of conveying reinforcing properties on otherwise neutral stimuli (Caggiula et al., 2001; Goldberg et al., 1981; Palmatier et al., 2007). Indeed, cigarette smoke produces distinct and salient sensory cues that are frequently and consistently paired with nicotine delivery and are thought to be subject to a conditioned (Pavlovian) association with nicotine delivery (Rose & Levin, 1991). Hence, smoking may be maintained by both the primary reinforcing effects of nicotine and the subsequent conditioned effects of nicotine-associated stimuli (in addition to other pharmacologically active constituents). Consequently, the drastic reduction of nicotine would be expected to have two effects. First, smoking behavior promoted by the reinforcing properties of nicotine should decrease. Second, the conditioned effects of stimuli associated with nicotine should

decrease over time as a consequence of extinction (i.e., repeated exposure to the conditioned stimulus in the absence of the unconditioned stimulus).

Effects of very low nicotine content (VLNC) cigarettes:
Studies of VLNC (e.g., 0.05 mg nicotine yield) cigarettes suggest that, acutely, they produce many effects in smokers that are qualitatively similar to normal nicotine content (NNC; e.g., 0.8 mg yield) cigarettes, but with somewhat reduced efficacy. VLNC cigarettes reinforce behavior (Shahan et al., 1999; Shahan et al., 2001), maintaining similar rates of self-administration as NNC cigarettes despite the fact that participants prefer NNC cigarettes when given a choice (Shahan et al., 1999). Compared to not smoking, VLNC cigarettes increase ratings of satisfaction and liking (Donny et al., 2007; Donny & Jones, 2009; Rose et al., 2000), although the magnitude of these effects is typically reduced compared to those produced by NNC cigarettes (Butschky et al., 1995; Gross et al., 1997; Robinson et al., 2000). VLNC cigarettes also reduce withdrawal and craving (Pickworth et al., 1999), although some symptoms (e.g., restlessness, impatience) may be more effectively alleviated by NNC cigarettes (Buchhalter et al., 2005). Much less is known about the effects of VLNC cigarettes over an extended period of use. When only VLNC cigarettes were available in an inpatient setting, the number of cigarettes smoked and the motivation to smoke during periods of abstinence decreased over time (Donny et al., 2007). Reductions in reinforcement in the real world, however, may prove somewhat more difficult. Outpatient smoking rate remained unchanged for a week after switching to VLNC cigarettes (Benowitz et al., 2007; Benowitz et al., 2009; Donny & Jones, 2009), but declined significantly over a period of 3-6 weeks (Hatsukami et al., 2010). During this time, participants also reported minimal withdrawal symptoms and a reduction in nicotine dependence as measured by the FTND (Hatsukami et al., 2010). These data suggest that the conditioned reinforcing effects of cigarettes can be extinguished, but that the process is on the order of weeks rather than days. Finally, it is important to note that there is little evidence to suggest that prolonged use of VLNC cigarettes will result in a compensatory increase in smoking. Data available to date indicate that smoking is first maintained at a similar rate compared to preferred brand and then decreases over time (Donny & Jones, 2009; Hatsukami et al., 2010). Furthermore, participants tend to reduce the volume of smoke inhaled and demonstrate a decrease in expired CO (Donny & Jones, 2009; Hatsukami et al., 2010). These findings are in contrast to reports indicating an acute compensatory increase in total puff volume in participants smoking VLNC cigarettes (Strasser et al., 2007). Hence, VLNC cigarettes may produce a short-lived compensatory increase in smoking, but this effect likely dissipates quickly and is replaced by a decrease in smoke intake. Nevertheless, the study proposed below will closely monitor puff topography and markers of exposure to continue to address concerns about possible compensatory use of VLNC cigarettes.

In the context of considering a policy for low nicotine standards for cigarettes, the optimal upper limit for nicotine yields per cigarette is one that results in decreased abuse liability and exposure to nicotine and other constituents of tobacco smoke. The upper limit is expected to be less than, but likely near, the level of nicotine that results in sustained use and dependence. Benowitz and Henningfield (1994) proposed a value of approximately 0.2 mg (0.17 mg) of nicotine per cigarette as a threshold yield for establishing and sustaining addiction. More recent data support this estimate. Smoking rate for reduced nicotine content (RNC) cigarettes (i.e., above 0.2 mg but below NNC) tends to persist at the same or a somewhat higher rate than NNC cigarettes (Donny et al., 2007; Donny & Jones, 2009; Hatsukami et al., 2010). In contrast, much lower nicotine yields (e.g., 0.05 mg) result in reduced use and dependence (Donny et al., 2007; Hatsukami et al., 2010). Indeed, a 40% reduction in smoking behavior was observed over a 6-week period of smoking VLNC cigarettes with minimal experience of withdrawal symptoms, a reduction in FTND scores, and reduced exposure to the potentially harmful byproducts of tobacco (Hatsukami et al., 2010).

Despite the evidence that nicotine yield cigarettes below 0.2 mg will reduce cigarette use and exposure to smoke constituents, little data are available that have directly addressed the effect of different nicotine yields near or below this estimate. No published study has examined yields less than 0.05 mg. It is reasonable to expect that lower values could result in similar or more rapid declines in use and dependence, but possibly at the cost of increased withdrawal and reduced acceptability/compliance. At the upper end, three studies have evaluated cigarettes with yields >0.07 and ≤0.20 mg (Benowitz et al., 2007; Benowitz et al., 2009; Benowitz et al., 2012). In two of the studies, 20 participants smoked cigarettes with decreasing nicotine yield over the course of 6 weeks (1 week per yield). The third study was similar in design but nicotine yields were decreased on a monthly basis. The yields evaluated started in the usual brand range (0.8-0.9 mg) and decreased through several intermediate steps to 0.2 then 0.1 mg. Results showed that although nicotine intake progressively declined, cigarette use remained stable throughout the assessment period (Benowitz et al., 2007; Benowitz et al., 2009, Benowitz et al., 2012). Given the relatively short duration of use, the lack of a decline in smoking is not surprising and consistent with other reports of even lower nicotine yield cigarettes (Donny & Jones, 2009; Hatsukami et al., 2010). Interestingly, one study observed a decrease in blood carboxyhemoglobin, total NNAL and polycyclic aromatic hydrocarbons at 0.1 mg (Benowitz et al., 2009) suggesting total smoke exposure was reduced, even if self-reported cigarettes per day were not. Furthermore, in both studies, nicotine dependence and subsequent use of preferred brand smoking decreased after the taper to 0.1 mg.

In sum, the available literature provides relatively little insight into the precise relationship between nicotine yield and potentially important outcomes in individuals smoking VLNC cigarettes over a prolonged period of time. The existing evidence supports the notion that nicotine yields <0.2 mg will likely produce the desired profile of effects, but additional information is needed.

Cigarettes to be assessed in this study:

The cigarettes to be used in this study were made under an NIH contract with production being overseen by the Research Triangle Institute (referred to as “Spectrum cigarettes”). NIH currently has approximately 10 million of these cigarettes (of varying types) for research purposes. The cigarettes selected for the study span the range of yields likely to produce the hypothesized effects, as described above. The Spectrum cigarettes are not currently commercially available, although they are similar in many ways to marketed cigarettes (e.g., similar manufacturing, filter, paper, etc.).

The 13 mg tar yield condition was added to the design because it is similar to full flavor cigarettes. Some researchers believe it will make the VLNC cigarettes more acceptable because it will replace some of sensory properties (i.e., not taste too light or too much like air). Some studies have shown that high tar in cigarettes with normal nicotine content may increase harm (e.g., lung cancer risk) when smoked over a long period of time. However, whether the combination of very low nicotine and high tar yield results in increased tar exposure relative to preferred brand is unknown. We believe the cigarettes to be used here are unlikely to lead to adverse health effects in these participants because of the short period of exposure, the low chance of continued use of these high tar cigarettes because they are not commercially available, and, importantly, because of the very low nicotine content (which will likely lead to reduced smoking).

Screening Procedures

Recruitment:
Participants will be recruited through flyers, direct mailings, television, radio, newspaper, bus, and Craigslist advertisements that read "Smokers who want to try new cigarettes that may or may not lead to reduced smoking are wanted for research. Participants will be paid for participation." Those who call into the laboratory will be read a script briefly explaining the study. After verbal informed consent is received, the participants will be asked questions over the phone to determine initial eligibility. If eligible and interested, they will be scheduled for an in-person screening interview. 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 state issued photo ID to the screening visit. Acceptable forms of identification include a Driver's License, State Photo ID Card, Passport, or Military ID. If the potential participant does not have a valid state issued 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, potential participants will need to produce valid, state issued photo identification. The interviewer will confirm the age and identity of the participant. If the participant is not age 18 or older, he/she 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 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 all written measures and materials to the participant for the remainder of the screening visit. The interviewer will use a PowerPoint presentation to discuss the procedures, risks and benefits with the participant as well as his/her rights as a research participant. At the end of the presentation, the participant will be instructed to read several open-ended questions aloud and discuss the answers with the researcher. Only after the participant and the researcher are fully satisfied that the participant understands the purpose of the study, the confidentiality of the data, the procedures, the risks/benefits and his/her 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:

The following physiological measures will be collected, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

1) Breath alcohol levels (BAL) will be measured 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 received 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. 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 Smokerlyzer ED50 CO meter (Bedfont Instruments), a reliable and valid measure of recent smoking.
    a. NicAlert Strips will be used to asses urinary cotinine levels if a participant's carbon monoxide reading is less than or equal to 8 ppm.
4) 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 may reschedule the interview but will need to be re-consented to ensure they have received adequate informed consent. They will be excluded if they are positive the second time.
5) Pregnancy Tests (HCG detection) will be performed for female participants with childbearing potential. We will also ask the date of last menstrual period and length of cycle.
6) Blood pressure and heart rate will be measured using a CritiCare monitor to help the licensed medical professional 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 at the end of the visit:

1) Identifying Information Form will include the participant's OnCore Subject Identifier, name, address (including the county of residence), email address, phone number, age, date of birth, and social security number.
    a. This form will be entered into the 'Identifying Information Access Database'.
        i. Each site will have a separate 'Identifying Information Access 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 Access Database (electronic version) separate from all other study data.
2) Brief Medical History Questionnaire to assess current diagnoses, symptoms and past health problems.
    a. Sections of the questionnaire will be entered into OnCore.
    b. The medications section will be transferred onto the 'Concomitant Medications' form and entered into OnCore.
3) Prime MD, a brief questionnaire developed for evaluation of mental disorders by primary care physicians (Spitzer et al., 1999).
    a. This questionnaire will be entered into Qualtrics.
4) Beck Depression Inventory (BDI; Beck, Ward, & Mendelson, 1961), 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 and then will be entered into the study databases by the interviewer at the end of the visit:

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 (Sheehan et al., 1997) 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 Qualtrics.
4) Drug Use Questionnaire (12 month and 1 month version)
    a. This questionnaire will be entered into Qualtrics.
5) Smoking Cessation Therapy Use Questionnaire to assess use of nicotine replacement therapy or smoking cessation medications to help participants quit smoking.
    a. This questionnaire will be entered into Qualtrics.

The following screening assessments will be 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 and 1 month version)
3) Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991)
4) Smoking Stages of Change Algorithm as well as a contemplation ladder to assess intention to quit smoking (DiClemente et al., 1991).

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Suicidality/Mental Health Monitoring

Participants who make any response other than "not at all" on the suicidal ideation question of the Prime MD (Question 1i) or indicate suicidal ideation or attempt in the past month or a suicide attempt in the past 10 years on the 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 (BDI) and refer to the licensed on-site clinician for evaluation. In the event that no clinician is available, staff will ask the participant two questions to determine level of risk: "Are you feeling suicidal?" and "Do you have a plan to kill yourself today?" If the participant has a plan to kill himself/herself, staff will put the participant in contact with the National Suicide Prevention Lifeline at 1-800-273-8255. If the participant refuses to talk to the hotline and leaves, the study staff will call 911. They will also contact the Study Coordinator and Site PI to inform them of the situation as soon as possible. Additionally, they will contact the Project Coordinator (Rachel Denlinger) to inform her of the situation. If the participant does not have a plan to kill himself/herself, the study staff will recommend he/she speaks with the suicide hotline and inform him/her that he/she will not be eligible for the study. The participant will be paid $25 and provided with local mental health resources.

Additionally, any participant whose score on the Prime MD indicates Major Depressive Disorder will be administered the Beck Depression Inventory (BDI) on paper. The BDI will be submitted, along with the Prime MD, Brief Medical History Questionnaire, Brief Medical History Follow-up Questionnaire, and the MINI suicide subscale to the licensed medical professional for eligibility review. If he/she determines a participant with Major Depressive Disorder is eligible for study participation, the participant will complete the BDI on a weekly basis to monitor changes in his/her mood.

Inclusion/Exclusion Criteria

Inclusion Criteria:

1) Age 18+
2) Smoke an average of at least five cigarettes per day for at least 1 year
3) Breath CO levels > 8 ppm (if ≤ 8 ppm, then NicAlert Strip > 2)

Exclusion Criteria:

1) Intention to quit smoking in the next 30 days
2) Currently seeking treatment for smoking cessation
3) Currently using nicotine replacement therapies or other pharmacotherapies as cessation aid (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 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) Significant 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 in the past 3 months including new prescriptions, changes in dosages or discontinuation of medications
10) Positive toxicology screen for any of the following drugs: cocaine, opiates, methadone, benzodiazepines, barbiturates, amphetamines, methamphetamines, and PCP
    a. Marijuana will be tested for but will not be an exclusionary criterion.
    b. Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines or methadone will not be excluded.
    c. Participants failing the toxicology screen will be allowed to re-screen once. These participants will need to be re-consented before being rescreened to ensure they have received adequate informed consent.
11) Breath alcohol level > 0.01
    a. Participants failing the breath alcohol screen will be allowed to re-screen once. These participants will need to be re-consented before being rescreened to ensure they have received adequate informed consent.
12) Binge drinking alcohol (more than 9 days in the past 30 days, 4/5 drinks in a 2 hour period (female/male))
13) Pregnant, trying to become pregnant or breastfeeding
14) Smoking 'roll your own cigarettes' exclusively
15) Currently taking anticonvulsant medications including:
    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
16) CO reading >80 ppm
17) Systolic BP greater than or equal to 160
    a. Participants failing for blood pressure will be allowed to re-screen once.

18) Diastolic BP greater than or equal to 100
    a. Participants failing for blood pressure will be allowed to re-screen once.
19) Systolic BP below 90
    a. Participants failing for blood pressure will be allowed to re-screen once.
20) Diastolic BP below 50
    a. Participants failing for blood pressure will be allowed to re-screen once.
21) Heart rate greater than or equal to 115 bpm
    a. Participants failing for heart rate will be allowed to re-screen once.
22) Heart rate lower than 45 bpm
    a. Participants failing for heart rate will be allowed to re-screen once.
23) Indicating any suicidal ideation in the past month or suicide attempts in the past 10 years.
24) Inability to independently read and comprehend the consent form and other written study materials and measures.
25) Having participated in a research study during the past three months in which the participant:
    a. Smoked a cigarette that was not his/her usual brand cigarette for more than one day
    b. Used any tobacco products beyond normal use for more than one day
    c. Used any nicotine replacement products or smoking cessation medications for more than one day

Children under age 18 are excluded because they cannot legally buy cigarettes. Those with unstable medical, psychiatric, or medication conditions (condition and/or medication changes in the past 3 months) 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, 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. 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. We will exclude pregnant and nursing women and anyone with current or recent alcohol or drug abuse problems 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 10 years will be excluded from the study for safety concerns. Individuals who smoke 'roll your own' cigarettes exclusively will be excluded from the study because we will be unable to standardize their baseline smoking behavior. Participants that are currently prescribed anticonvulsant medication will be excluded because this medication can interfere with the biomarkers. If an individual has recently participated in a smoking research study that changed his/her smoking behavior this person would be excluded because he/she would not have a stable smoking baseline. Because participants are required to complete portions of the protocol independently both in the lab and at home, they will need to be able to independently read and comprehend the study materials.

Eligibility Determination:
The final eligibility of the participant will be determined by a licensed medical professional (MD, DO, NP, PA or CRN) 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 licensed medical professional for review

as well. Additionally, if the participant's score on the Prime MD indicates Major Depressive Disorder, then the Prime MD along with the Beck Depression Inventory will be submitted 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 licensed 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 prior to the first baseline visit. The licensed 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 he/she is taking, then he/she will not be automatically excluded. The interviewer will record the positive result into OnCore and perform an eligibility override to prevent the system from marking the participant as ineligible. The interviewer will make note of this when he/she submits the forms to the licensed medical professional for final eligibility determination.

Once all the screening procedures have been completed, researchers will pay participants $25 for their time as long as they pass the drug and breath alcohol tests and meet the minimum requirements for carbon monoxide or NicAlert levels. Those participants who do not pass these tests or meet these requirements will be dismissed from the study without payment. Marijuana will be tested for but will not be an exclusionary criterion. If a participant does not pass the drug test but has a current, valid prescription that would explain the failed test he/she will not be automatically excluded and will still receive $25. Participants who meet all other eligibility criteria, sans the medical criteria, will be scheduled for the first baseline visit.

At the end of the screening session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, 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, 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 significant medical problems including:
    a. Cardiovascular Diseases: Coronary heart disease, heart attack, stroke, peripheral vascular disease, reduced blood circulation, abdominal aortic aneurysm
    b. Respiratory Diseases: Emphysema, bronchitis, and chronic airway obstruction

c. Cancers: Cancer of the lung, bladder, cervix, esophagus, kidney, larynx, mouth, pancreas, throat, and stomach; leukemia
d. Other Health Risks Associated with Smoking: Including but not limited to infertility, lower bone density in postmenopausal women, and hip fracture in women
e. Death

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. Due to the altered nicotine levels, there could be a change in their cigarette use including the manner in which they inhale the smoke. Smoking the study cigarettes does not provide any less risk than their usual brand cigarette and could pose increased health risks. Participants may also experience increases in levels of carbon monoxide, a gas from smoke.
7) Smoking Withdrawal: Participants may experience smoking withdrawal symptoms during this study. The symptoms can be uncomfortable but are typically of minimal risk. 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 cigarette at the end of the study they may experience mild and transient nausea, dizziness, and lightheadedness.
9) Risk to Fetus**:** Smoking during pregnancy can lead to 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 cigarettes consumption could adversely affect psychiatric conditions

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 screening visit, before randomization during the Baseline 2 visit and at the Week 6 visit. If a participant becomes pregnant during the study, she will be withdrawn from the study. Approximately 30 days after being withdrawn or having a positive pregnancy test at the Week 6 visit, the research staff will call 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.

Baseline Procedures

This study will use a one 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, reduce reactivity to the daily cigarette monitoring, and reduce participant burden. During the two baseline sessions, participants will complete subjective questionnaires, assessments of cognitive functioning, and smoking topography. Each visit will last approximately two hours or less. At the end of each baseline session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, and to assess the truthfulness of the participant in regards to self-report of tobacco use.

Visit scheduling requirements for baseline period:
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/she will need to be re-screened. This will be entered into OnCore as an 'Unscheduled Visit'. The participant will need to be re-consented but will maintain the original OnCore Subject Identifier. The ideal target window separating Baseline 1 and Baseline 2 is between 6 and 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 he/she will not be rescheduled and will be discontinued from the study.

Measures/Assessments

Physiological measures collected at Baseline 1, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

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

Physiological measures collected at Baseline 2, recorded on paper, and entered into OnCore by the interviewer at the end of the visit:

1) BAL

2) Weight
3) Three CO readings (initial visit CO, pre-topography and post-topography)
4) Blood Pressure
5) Heart Rate
6) Urine Pregnancy Test, if applicable

The following assessments will be administered as an interview and then entered into the study databases by the interviewer at the end of the visit:

1) Concomitant Medications Form
    a. This form will be entered into OnCore.
2) Medical Event Form, if applicable, will assess the nature, severity, duration, action taken, and outcome of medical event.
    a. This form will be entered into OnCore.
3) Health Changes Questionnaire which will assess any weekly health changes.
    a. This assessment will be entered into Qualtrics.
4) Timeline Follow Back Questionnaire**,** which will assess other tobacco and nicotine product use as well as alcohol and marijuana use during the past 14 days. (Baseline 2 only)
    a. This assessment will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source documents only:

1) BDI, if applicable

The following assessments will be administered using Qualtrics in order to standardize assessments across sites and projects:

Baseline 1 Only:

1) Wisconsin Index of Smoking Dependence Motives-Brief (WISDM; Piper et al., 2008), a measure of tobacco dependence
2) Perceived Health Risks Rating (Hatsukami et al., 2010), a measure of the perceived addictive potential and other health risks associated with cigarettes
3) Perceived Stress Scale - 4 item (PSS-4; Cohen, Kamarck, & Mermelstein, 1983), which measures the degree to which life situations are appraised as stressful
4) Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegan, 1988), which measures symptoms of positive and negative affect.
5) Center for Epidemiological Studies-Depression Scale (CES-D; Radloff, 1977), which measures symptoms of depression
6) Short Michigan Alcohol Screening Test (SMAST; Selzer et al., 1975), which measures past alcohol use
7) Drug Abuse Screening Test Brief Version (DAST-10; Gavin, Ross, & Skinner, 1989), which measures prior drug use and abuse

Baseline 1 & 2:

1) Respiratory Health Questionnaire, a measure of cough, shortness of breath and other respiratory symptoms
2) Minnesota Nicotine Withdrawal Scale (MNWS; Hughes & Hatsukami, 1986), a measure of nicotine withdrawal
3) Questionnaire of Smoking Urges-brief scale - Usual Cigarette (QSU; Cox, Tiffany, & Christen, 2001; Tiffany & Drobes, 1991), which measures the urge to smoke

4) Cigarette Evaluation Scale (CES; Westman, Levin, & Rose, 1992), which measures responses to cigarettes (e.g., reward, satisfaction)

Baseline 2 Only:

1) Environmental and Social Influences on Tobacco Use Questionnaire (adapted from Nondahl, Cruickshanks, & Schubert, 2005), which measures tobacco smoke exposure at home, work and socially
2) Cigarette Purchase Task – Usual Brand Version (Jacobs & Bickel, 1999; MacKillop et al., 2008) 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 subject reports zero consumption) and Pmax (the price at which Omax occurs). This task will indicate whether prolonged VLNC cigarette 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.

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Interactive Voice Response System:

Participants will also 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 as well as withdrawal symptoms the week before and after randomization. Participants will be provided a study cell phone if they have unreliable telephone access, do not have enough monthly cell phone minutes or prefer not to use their own phone.

The IVR system is operated by InterVision Media, a media production company. To be enrolled in the IVR system, research staff will enter the participant's initials, telephone number, subject identifier, and visit dates into the IVR CENIC website. Identifying information (initials and telephone numbers) will not be extracted with the data by the bioinformatics group. Please refer to InterVision Media's privacy statement and HIPAA compliance form for additional information.

Cognitive Tasks (Baseline 2 Only)

Cognitive functioning will be assessed using a battery of computer-based assessments. We will assess domains that are theoretically linked to smoking and likely to be sensitive to nicotine abstinence (Heishman, 1999; Kleykamp et al., 2005; Rycroft et al., 2006). Prior to test administration, participants will be trained to ensure their understanding of each test. Tests will be administered on a desktop computer.

1) **N-Back (0,2) Task** (Ernst et al., 2001): A measure of working memory in which participants view serially presented letters on a computer. They must indicate whether each letter presented is the same or different from the letter presented a specified number of positions back in the string of letters (e.g. 2-back).
2) **2-Letter Search** (Ernst et al., 2001): A measure of focused attention in which participants view strings of letters on a computer screen looking for whether each string contains or does not contain two target letters.

3) **Continuous Performance Test** (CPT; Myers et al., 2008): A measure of sustained attention, participants must monitor a string of stimuli (e.g. letters) serially presented on a computer screen monitoring for presentation of a target stimulus. The task is balanced so that they either must respond, or inhibit a response each time the target is presented.
4) **Heishman Arithmetic Test** (Myers et al., 2008): A measure of information processing speed and basic arithmetic. Participants view solved single digit addition or subtraction equations presented on a computer screen and must indicate, as quickly as possible, whether the solution provided is correct or incorrect.

Puff Topography (Baseline 2 Only)

Puff Topography, a precise measure of smoking behavior (Brauer et al., 1996; Herning et al., 1981; Robinson & Forbes, 1975), will be used to examine whether prolonged use of the experimental cigarettes affects topography measures that may indicate smoking compensation (Strasser et al., 2007). Puff topography will be assessed using a handheld topography device that provides a valid measurement of puff number, puff volume, inter-puff interval and other indices (Blank et al., 2009). Carbon monoxide readings will be collected before and 15 minutes after puff topography. The cigarette butt will be collected in a freezer vial, stored at room temperature, and sent to the CDC for solanesol analysis.

Biological Samples

Baseline 1 biological specimens collected, stored, and entered into caTissue:

1) Saliva sample for DNA analysis**:**
   Participants will be asked to provide a saliva sample for DNA analysis. The sample will be collected using an OraGene kit. Saliva samples will be sent quarterly to be stored at the University of Minnesota. The DNA sample will be used to determine if there are links between genes and levels of tobacco constituents, behavior, mood, brain functioning, the harmful effects of smoking. Participants who chose not to provide a DNA sample will still be allowed to participate in the study.

Baseline 2 biological specimens collected, stored, and entered into caTissue:

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 stored at temperatures no more than -20°C. Urine samples will be sent quarterly to be analyzed and stored at the University of Minnesota. The tobacco-specific carcinogen biomarkers are total NNAL and total NNN. We will also assess total nicotine equivalents and minor alkaloids, including anatabine, anabasine, myosmine, and nornicotine. Platelet activation will be assessed with the cardiovascular biomarker 11-dehydroTXB2. If a participant forgets to bring his/her urine sample, then an on the spot urine sample will be collected.
2) Saliva sample for cotinine assessment:
   Participants will be asked to provide two saliva samples during the second baseline session for assessment of nicotine metabolite ratio (NMR), an indicator of CYP2A6 enzyme activity. Participants must wait 30 minutes after arrival to the lab before collecting the first saliva sample. During this time participants cannot eat, drink, chew gum or smoke cigarettes. After collecting the sample, provide time for the participant to eat and/or drink before waiting another 30 minutes before collecting the second saliva sample. The second saliva sample must be collected prior to puff topography. Samples will be stored at temperatures no more than -20°C. Saliva samples will be sent quarterly to be analyzed and stored at the University of Minnesota.

Baseline 2 biological specimens collected, stored, and shipped to the CDC:

1) Cigarette butt from puff topography:
   The cigarette butt will be collected in a freezer vial. 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.
2) 24-hour cigarette butt collection:
   Cigarette butts will be collected during the 24 hours prior to the biosamples first void urine collection. Participants will be provided with 20 tins and a collection box. They will be instructed to record the time each cigarette was smoked and put the cigarette butts into the collection box in the order they were smoked. Both usual brand and non-usual brand 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.

Study Procedures

Randomization

Following baseline, participants will be randomized into one of seven cigarette conditions. Participants in each condition will be assigned a cigarette that matches their menthol preference.

Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
1	NRC600	CN	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
1	NRC601	CN-Men	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
2	NRC400	RN	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
2	NRC401	RN-Men	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
3	NRC300	RN	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
3	NRC301	RN-Men	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
4	NRC200	RN	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
4	NRC201	RN-Men	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
5	NRC102	RN	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
5	NRC103	RN-Men	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
6	NRC104	RN-HT	0.04 ± 0.02	13 ± 2	0.02 - 0.06
6	NRC105	RN-HT-Men	0.04 ± 0.02	13 ± 2	0.02 - 0.06

Non-Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
7	N/A	USUAL BRAND	N/A	N/A	N/A
7	N/A	USUAL BRAND - Men	N/A	N/A	N/A

*Legend:	
RN-HV	Reduced Nicotine-High Ventilation
RN-HV-Men	Reduced Nicotine-High Ventilation Menthol
RN	Reduced Nicotine
RN-Men	Reduced Nicotine-Menthol
RN-HT	Reduced Nicotine-High Tar
RN-HT-Men	Reduced Nicotine-High Tar-Menthol
CN	Conventional Nicotine
CN-Men	Conventional Nicotine-Menthol
CN-HT-Men	Conventional Nicotine-High Tar-Menthol
LTNR	Low Tar/Nicotine Ratio
LTNR-Men	Low Tar/Nicotine Ratio-Menthol

The Administrative Core for the Center for the Evaluation of Nicotine in Cigarettes (CENIC) will be responsible for removing all identifying information from cigarettes received from the Research Triangle Institute (RTI), labeling each carton with a blind code, assigning product using this blind code based on the randomization schedule being provided by the CENIC Biostatistics Core, and shipping cigarettes to each site as needed based on recruitment. Each site will be responsible for tracking product received and distributed to participants, collecting unused product from participants, and destroying unused open packs. The participants, investigators and study staff will not have knowledge of which product is given to a participant or whether different participants received the same or different product.

During the experimental period, participants will be provided with a 14-day supply of research cigarettes or their usual brand cigarettes. 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. Participants will be instructed to use the research cigarettes for 6 weeks, at which point they are to discontinue product use.

If there is prior knowledge a participant will be missing a visit (i.e. planned vacation, laboratory closure, etc.), then the participant will be provided with an adequate supply of cigarettes to make up for the missed visit(s). The participant will be given a 21 day supply if one visit is going to be missed and a 28 day supply if two visits are going to be missed.

Participants will be asked to refrain from use of other non-study cigarettes during the study period. However, they will be told there is not a penalty for use of non-study cigarettes, and that it is crucial for them to report any use of non-study cigarettes or other nicotine or tobacco products. Throughout the baseline and experimental periods, an Interactive Voice Response (IVR) system will be used on a daily basis to record the number of study cigarettes and non-study cigarettes used the previous day. During the baseline and first experimental week, participants will also answer daily IVR questions about their mood. Participants will be seen weekly for assessments. Brief standardized review sessions focusing on compliance with the study cigarettes and other study procedures will be provided at each visit. At the

end of the 6-week trial, participants will undergo an assessment of withdrawal, craving, and cognitive function following a brief period of abstinence.

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 laboratory each week. Research staff will complete 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. Unused cigarettes will be re-distributed to the participants during Weeks 1-5. During Week 6, any remaining unused cigarettes returned by the participants will be collected by the research staff.

If participants lose more than two packs of cigarettes and require an unscheduled visit to the laboratory to supplement their supply, they will be told the next time they lose more than two packs they will have to wait until their next scheduled appointment to receive more cigarettes.

Experimental Period:
Participants will be seen weekly throughout the 6-week experimental period. Weeks 2 and 6 will take approximately 1-2 hours each. All other sessions will last 1 hour or less. At the end of each experimental session, the researcher will complete the End of Visit Evaluation Form, which will be entered into OnCore. This will allow the researcher to make note of any problems encountered during the visit, to track which computers were used for which tasks, and to assess the truthfulness of the participant in regards to self-report of tobacco use and compliance to study procedures.

Visit scheduling requirements for experimental period:
OnCore will automatically generate the ideal visit calendar once the participant has been put 'On Study'. The perfect scheduling window between each visit is 7 days based on the date of the Baseline 2 Visit. For additional scheduling requirements, refer to the '*Scheduling Visits SOP*'. If a participant misses a visit and is not able to reschedule during the window, that visit will not be 'made-up' in the future. All measures that were not completed will be considered missing data and will not be collected during future visits. If a visit mistakenly occurs outside of the designated window, this is a protocol deviation. A 'Non-Medical Event Form' will need to be completed and tracked in OnCore.

If a participant is not able to attend his/her Week 6 visit, then it should be rescheduled even if it is outside of the scheduling window. This will be documented as a protocol deviation.

Experimental Sessions 1, 3, 4, and 5 Procedures

Measures/Assessments

Physiological Measures Collected, recorded on paper, and entered into OnCore by interviewer at the end of 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 the study databases by the interviewer at the end of the visit:

1) Concomitant Medications
    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow Back Questionnaire
    a. Will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source document only:

1) BDI, if applicable

The following assessments will be administered using Qualtrics:

1) Respiratory Health Questionnaire
2) MNWS
3) QSU brief - Usual Brand Cigarette
4) QSU brief– Study Cigarette
5) Cigarette Evaluation Scale

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Experimental Sessions 2 and 6 Procedures:

Measures/Assessments

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

1) BAL
2) Weight
3) Three CO readings (initial visit CO, pre-topography and post-topography)
4) Blood Pressure
5) Heart Rate
6) Urine sample for drug test and pregnancy test (if applicable) (Week 6 only)

The following assessments will be administered as an interview and will be entered into the study databases by the interviewer at the end of the visit:

1) Concomitant Medications
    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow Back Questionnaire
    a. Will be entered into Qualtrics.

5) Drug Use Questionnaire - 1 month version (week 6 only)
    a. Will be entered into Qualtrics.

The following assessment will be administered on paper and kept as a source documents only:

1) BDI, if applicable

The following assessments will be administered using Qualtrics:

1) Respiratory Health Questionnaire
2) MNWS
3) QSU brief - Usual Cigarette
4) QSU brief – Study Cigarette
5) FTND
6) Cigarette Evaluation Scale
7) Perceived Health Risks Questionnaire
8) Smoking Stages of Change Algorithm and Contemplation Ladder
9) Cigarette Purchase Task - Usual Brand Cigarette Version
10) Cigarette Purchase Task – Study Cigarette Version
11) PANAS
12) Perceived Stress Scale – 4 item
13) Alcohol Use Questionnaire -1 month version (week 6 only)
14) Environmental and Social Influences on Tobacco Use Questionnaire (week 6 only)
15) CESD (week 6 only)
16) WISDM-Brief (Week 6 only)

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Participants will also complete the following tasks:

1) Cognitive tasks
2) Puff Topography

<u>Biological Samples</u>

Biological specimens collected, stored, and entered into caTissue:

1) First void urine sample

Biological specimens collected, stored and shipped to the CDC:

1) Cigarette butt from puff topography
2) 24-hour cigarette butt collection

Urine sample for smoking biomarker assessment:

Participants will be asked to bring a urine sample (first void of the day) to the week 2 and 6 sessions for biomarker assessment. Samples will be stored at temperatures no more than -20°C. Urine samples will be sent quarterly to be analyzed and stored at the University of Minnesota. The tobacco-specific carcinogen biomarkers are total NNAL and total NNN. We will also assess total nicotine equivalents and minor alkaloids, including anatabine, anabasine, myosmine, and nornicotine. Platelet activation will be

assessed with the cardiovascular biomarker 11-dehydroTXB2. If a participant forgets to bring his/her urine sample, then an on the spot urine sample will be collected.

Cigarette butt from puff topography:
The cigarette butt will be collected in a freezer vial. 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.

24-hour cigarette butt collection:
Cigarette butts will be collected during the 24 hours prior to the biosamples first void urine collection. Participants will be provided with 20 tins and a collection box. They will be instructed to record the time each cigarette was smoked and 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 experimental period to record the number of study cigarettes smoked per day and use of non-study cigarettes. During the first week after randomization, the IVR system will collect information about withdrawal symptoms.

Product and Procedures Compliance Review Sessions:
At each visit, Baseline 2 through Week 5, participants will be counseled about their use of the study cigarettes. Participants will be asked about any concerns or obstacles associated with use of the study cigarettes. The importance of honest self-reporting will be stressed. Participants will be told that they will not be penalized for use of other nicotine or tobacco products and 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. Additionally, participants will be counseled about their IVR completion, visit attendance, task engagement and product accountability. Refer to the '*Product and Procedures Compliance Review Sessions SOP*' for more information.

Quit Attempts During the Study Protocol:
At each weekly session, we will ask the participant if he/she is currently abstaining from smoking with the intention of quitting. If the answer is no, then we will also ask if he/she is planning to quit smoking prior to his/her next scheduled visit.

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 visits, but no puff topography
- 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 choses 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 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 that 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 as to avoid unwanted cues to smoke.

Abstinence Assessment Session:

After the week 6 visit, participants will be required to come back for one additional visit the following day. During this visit, participants will have been encouraged to abstain from smoking until their next scheduled visit (approximately 24 hours later). The abstinence assessment session should be scheduled no less than 18 hours and no more than 30 hours after the Week 6 visit. Abstinence will be verified by expired breath carbon monoxide levels that have decreased by at least 50% from the measure taken during the Week 6 visit (post-topography CO) or less than 6 ppm. This session will allow us to determine whether the experimental cigarettes have reduced the effects of abstinence on these measures relative to the control conditions. If the participant does NOT meet abstinence criteria, he/she will not receive the $90 for the visit.

Measures/Assessments

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

1) BAL
2) CO
3) Blood Pressure
4) Heart Rate

The following assessments will be administered as an interview and will be entered into study databases by the interviewer at the end of the visit:

1) Concomitant Medications
    a. Will be entered into OnCore.
2) Medical Event Form, if applicable
    a. Will be entered into OnCore.
3) Health Changes Questionnaire
    a. Will be entered into Qualtrics.
4) Timeline Follow-Back
    a. Will be entered into Qualtrics.

The following assessments will be administered using Qualtrics:

1) MNWS
2) QSU-brief - Usual Cigarette
3) QSU-brief - Experimental Cigarette
4) Cigarette Purchase Task - Usual Brand Cigarette Version
5) Cigarette Purchase Task - Study Cigarette Version

In the event that the Qualtrics website is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's binder. The interviewer will enter the data into Qualtrics when it resumes functioning properly. This information should be recorded in the 'End of Visit Evaluation Form' and entered in to OnCore.

Participants will also complete the following task:

1) Cognitive tasks

Participants who do NOT meet abstinence criteria will be required to complete the following assessments:

1) BAL
2) CO
3) Blood Pressure
4) Heart Rate
5) Concomitant Medications
6) Health Changes Questionnaire
7) Medical Event Form, if applicable
8) Timeline Follow-Back

Participant Compensation:

Participants will receive $25 for completing the screening visit, regardless of enrollment as long as the participant passes the drug test, breath alcohol test, and meets the minimum requirements for carbon monoxide or NicAlert levels. Participants who do not pass these tests will be dismissed from the screening visit without payment, except in the event they can produce a prescription for the medication that caused them to fail the drug test.

- Screening Session: $25
- Short sessions: $25 per session ($125 total)
- Long sessions: $75 per session ($225 total)
- Abstinence Session: $90 (meets abstinence criteria of less than 6 ppm and/or a 50% reduction from Week 6 CO and self-reports not smoking any cigarettes since the Week 6 visit)
- Completion and on time bonus: $200 (attends all 10 visits)
- IVR compliance: up to $160
- 30 day follow up phone call: $10
- Total: $835

Participants are free to discontinue at any time and will receive compensation for the sessions completed at the same rate listed above. However, they will not receive the completion bonus.

End of Study:

After a participant has completed all study procedures and has been paid for participation the research assistant should read the following script and give the participant the *Clearing the Air Manual.*

"Before you go, we want to encourage you to try to continue to be abstinent as long as possible. Although the study is over and abstinence is not required, you may find it easier to quit as a result of your participation. We would like to provide you with some resources should you decide to try to abstain from smoking (give "Clearing the Air" and hotline information). Please also feel free to consult with your physician and use any medications he/she deems appropriate. We will call you in approximately 30 days

to ask about your smoking since leaving the study. There is no right answer and we know how difficult quitting can be. Please just answer honestly. The call will take less than 5 minutes, and we will compensation you for your time by giving you another $10. Thanks again for your participation."

The following assessments will be administered using Qualtrics:

1) End of Study Questionnaire
2) Study Evaluation Questionnaire

30 Day Follow up Phone Call:

Participants will receive a follow-up phone call between 25 and 35 days after the abstinence assessment session to assess their smoking patterns. The phone questionnaire will last less than five minutes. The questionnaire will ask if the participant is still smoking, how much and whether he/she has attempted to quit smoking since the end of the study. Additionally, any Medical Event Forms that remain open from the last session will be discussed. If the participant became pregnant during the study, this would have been recorded as a medical event. During this phone call, the research assistant will confirm her due date. This event will remain open until delivery. At that time the licensed medical professional will contact the participant to ask a few questions about the baby's health and will update the Medical Event Form.

Once a participant has completed all study procedures and all open events have been closed, the PI will review the participant's binder and sign a form indicating study completion for that participant.

Study Debriefing:

After data collection is complete, participants will receive 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 Bioinformatics Core for at least 7 years after study completion.

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.
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 participant indicates she is pregnant or has a positive pregnancy test at Week 2, she will be withdrawn from the study, and this event will remain open until delivery. At that time the licensed medical professional will contact the participant to ask a few questions about the baby's

health and will update the open 'Medical Event Form'. A positive pregnancy test at Week 6 will trigger a 'Medical Event Form' to be completed but will not result in withdrawal since she is no longer receiving study product.

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.
    c. Note: If the second consecutive visit is the Week 6 session, then the participant would not be withdrawn from the study.

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

1) Cigarettes per day increase: Continued participation will be evaluated by the site PI 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 115 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 out of range, a 'Blood Pressure and Heart Rate Symptom Checklist' and 'Medical 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 'Medical 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 PI 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 PI can withdraw him/her from the study at the PI's discretion.

6) If a participant fails to attend his/her Baseline 2 Visit (Randomization) within the 21 day allowable visit window, he/she will not be eligible to reschedule this visit or continue participation in the study.

Investigational Tobacco Product

The Co-Directors of this Center grant, Drs. Donny and Hatsukami, have received an Investigational Tobacco Product (ITP) application to the FDA to cover the experimental cigarettes being used in this study. This application encompasses all Project 1 sites.

Certificate of Confidentiality

To help protect the participant's privacy, the Co-Directors of Center grant, Drs. Donny and Hatsukami, have received a Certificate of Confidentiality from the National Institutes of Health. With this certificate, the researchers cannot be forced to disclose 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 themselves 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 Outcome Variables:

- Number of cigarettes smoked per day
  - Study cigarettes
  - Combined study and non-study cigarettes

Secondary Outcome Variables:

- Measures of compliance: non-study cigarette use, drop-out rate
- Measures of discomfort/dysfunction: MNWS, PANAS, QSU, PSS-4, CESD
- Measures of other health-related behaviors: breath alcohol, urine drug screen, TLFB-marijuana use, Alcohol Use Questionnaire, Drug Use Questionnaire, DAST, SMAST, weight
- Measures of nicotine/tobacco dependence: FTND, WISDM
- Measures of tobacco exposure: CO, total nicotine equivalents, NNN, NNAL, minor alkaloids
- Measures of smoking context: Environmental and Social Influences on Tobacco Use
- Measures of intention to quit: Stages of Change, Contemplation Ladder

- Measures of compensatory smoking: puff topography, filter analysis
- Measures of other tobacco use: TLFB-other tobacco
- Measures of cigarette characteristics: CES, Cigarette Purchase Task
- Measures of cognitive function: N-back, 2-Letter Search, CPT, Arithmetic Task
- Measures of cardiovascular function: heart rate, blood pressure, urine 11-dehydroTXB2
- Measures of perceived risk: Perceived Health Risk Questionnaire

Statistical Approach

The primary objective of this trial is to evaluate the effects of cigarettes varying in nicotine content. Our general approach to statistical analysis will be as follows. First, we will compare the four primary experimental conditions, conditions (2) – (5), to the primary control condition, condition (1), for the number of cigarettes smoked per day at six weeks. Comparisons with conditions (6) and (7) will provide further insight into the impact of tar yield and product switching.

(1) Baseline characteristics including demographics and smoking history will be compared between the treatment groups to identify any baseline imbalances after randomization. Discrete variables will be summarized by frequencies and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate. Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA.

(2) Continuous outcomes will be summarized by the mean, standard deviation, median and range, while categorical outcomes will be summarized by frequencies and percentages. Skewed continuous outcomes will be log-transformed or square root-transformed as appropriate; for example we conventionally analyze cotinine on log scale.

(3) We expect groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis for all endpoints will only adjust for the baseline value of that endpoint (for precision). However, a secondary analysis will be completed adjusting for age, sex and race, along with any other covariates that differ across treatment groups at baseline with a p-value less than 0.20.

(4) All analyses will be completed using SAS (version 9.2 or 9.3) or R. P-values less than 0.05 will be considered statistically significant with the exception of the analysis of our primary analysis, where p-values less than 0.0125 will be considered significant after a Bonferroni multiple comparisons adjustment.

Our primary endpoint, cigarettes per day at 6 weeks, will be summarized by treatment group and analyzed using linear regression adjusting for cigarettes per day at baseline. We will first compare the primary experimental conditions, conditions (2) – (5), to the primary control condition, condition (1). P-values less than 0.0125 will be considered significant. A pairwise comparison will be completed among experimental conditions that are significantly different than from the control condition using an appropriate Bonferroni adjustment. A secondary analysis of the primary endpoint will be completed adjusting for age, sex and race, along with any other covariates that differ across treatment groups at baseline with a p-value less than 0.20. Finally, cigarettes per day will be analyzed using a linear mixed model to evaluate trends in the difference in the number of cigarettes per day over time.

Secondary endpoints will be analyzed following the same approach as the primary endpoint. The primary analysis of our secondary endpoints will use linear regression and adjust only for the baseline value.

Secondary analyses will consist of an adjusted analysis and a repeated measures analysis using a linear mixed model. In addition, we will also complete pre-planned subgroup analysis by sex (men vs. women), race (white vs. black) and menthol status (non-menthol versus menthol).

Power Analysis

Power analyses are based on two-sided, two-sample t-tests at the 1.25% level to control for multiple comparisons of VLNC cigarettes to NNC controls (4 nicotine yields; 4x1.25 = 5% level). With 96 completers per group (based on 20% attrition over the 6 week period), we will achieve 80% (90%) power to detect an effect size of 0.486 (0.550) which is less than the effect of VLNC vs. NNC cigarettes observed by Hatsukami et al. (2010) for cigarettes per day (CPD; 0.991), cotinine (2.096), FTND (0.603), and withdrawal (0.512). Given equal recruitment of males and females, we will also be able to detect an effect size of 0.693 (0.785) for analyses focused on a single sex (n=48/group).

OnCore Subject Identifier

The subject identifier is an alpha-numeric combination. Example: A001 would be University of Pittsburgh's first subject.

Site Identifier:
A = University of Pittsburgh
B = University of Minnesota Masonic Cancer Center
C = Brown University
D = Johns Hopkins University
E = University of Pennsylvania
F = Duke University
G = University of Texas MD Anderson Cancer Center
H = University of California, San Francisco
I = University of Minnesota Medical School Duluth
J = University of South Florida Moffitt Cancer Center

Subject ID:
001-899

OnCore 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
05= Week 5 clinic visit
06= Week 6 clinic visit
07= Week 6 + day 1 lab session (Abstinence Session)
30= 30 day follow-up phone call
99= UNSCHEDULED/ADDITIONAL visit

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P1S1 Protocol Amendments

Update #1: 4/16/13

- Added information regarding identifying information (participant initials and telephone number) provided to InterVision Media for IVR calls.
    - See page 16 of final protocol.

Update #2: 6/18/13

- Updated Exclusion Criteria for Systolic BP below 90, Diastolic BP below 50, and Heart rate lower than 45 bpm. The participant no longer needs to be symptomatic to be excluded.
    - See page 11 of final protocol – items 19, 20, and 22.
- Updated the Medical Monitoring criteria for Systolic BP below 90, Diastolic BP below 50, and Heart rate lower than 45 bpm. The participant no longer needs to be symptomatic for a medical event form to be completed.
    - See page 27 of the final protocol.

Update #3: 11/11/13

- Updated the Psychiatric Medication Changes Exclusion Criteria to clarify a change as new prescriptions, changes in dosages, or discontinuation of medications.
    - See page 10 of the final protocol – item 9.
- Updated the Anticonvulsant Exclusion Criteria to include the specific anticonvulsants that interfere with the Biomarkers.
    - See page 10 of the final protocol – item 15.
- Added information about providing additional study product for planned missed visits.
    - See page 19 of the final protocol.
- Updated the Abstinence Session criteria for participant compensation to include self-reported abstinence. Now the participant must self-report not smoking since the Week 6 visit and meet the CO criteria to earn the $90.
    - See page 25 of the final protocol.
- Clarified that subjects will not automatically be withdrawn from participation during the Week 6 visit if their CO and CPD have increased for the second week in a row since no additional cigarettes will be dispensed
    - See page 27 of the final protocol. - item 7c.

Update #4: 4/1/14

- Added the 'Study Evaluation Questionnaire' to complete right after participants complete the 'End of Study Questionnaire'
    - See page 26 of the final protocol.

Project 1, Study 1:

Investigating the Impact of Nicotine Using Spectrum Cigarettes

Statistical Analysis Plan

4/5/2013

Table of Contents

1. Introduction

This document will serve as the Statistical Analysis Plan for CENIC Project 1, Study 1. This document describes the planned statistical analysis for evaluating the dose-response relationship for very low nicotine content (VLNC) cigarettes. Details for the proposed analysis of the primary, secondary and exploratory endpoints are provided.

2. Trial Objectives

Project 1, Study 1 will evaluate the relationship between nicotine yield of VLNC cigarettes and cigarettes smoked per day, nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. In addition, we will also evaluate product compliance for the various yields of VLNC cigarettes.

3. Trial Design

This is a randomized, multi-center, double-blind study design. 840 subjects will be randomized to one of seven conditions. Participants in each condition will be assigned a cigarette that matches their menthol preference. The groups are as follows:

Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
1	NRC600	CN	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
1	NRC601	CN-Men	0.8 ± 0.15	9 ± 1.5	0.65 - 0.95
2	NRC400	RN	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
2	NRC401	RN-Men	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
3	NRC300	RN	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
3	NRC301	RN-Men	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
4	NRC200	RN	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
4	NRC201	RN-Men	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
5	NRC102	RN	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
5	NRC103	RN-Men	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
6	NRC104	RN-HT	0.04 ± 0.02	13 ± 2	0.02 - 0.06

6	NRC105	RN-HT-Men	0.04 ± 0.02	13 ± 2	0.02 - 0.06

Non-Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
7	N/A	USUAL BRAND	N/A	N/A	N/A
7	N/A	USUAL BRAND - Men	N/A	N/A	N/A

*Legend:	
RN-HV	Reduced Nicotine-High Ventilation
RN-HV-Men	Reduced Nicotine-High Ventilation Menthol
RN	Reduced Nicotine
RN-Men	Reduced Nicotine-Menthol
RN-HT	Reduced Nicotine-High Tar
RN-HT-Men	Reduced Nicotine-High Tar-Menthol
CN	Conventional Nicotine
CN-Men	Conventional Nicotine-Menthol
CN-HT-Men	Conventional Nicotine-High Tar-Menthol
LTNR	Low Tar/Nicotine Ratio
LTNR-Men	Low Tar/Nicotine Ratio-Menthol

Condition (1) will serve as the primary control condition, with conditions (2) – (5) serving as the primary experimental conditions. Condition (6) is an exploratory condition to determine the impact of varying the tar content on the acceptability of VLNC cigarettes, while condition (7) will serve as a second control condition to determine the impact of switching brands on the number of cigarettes smoked per day.

Subjects will be enrolled at 10 study centers (84 subjects per center): University of Pittsburgh, University of Minnesota Masonic Cancer Center, Johns Hopkins University, Brown University, University of Pennsylvania, Duke University, University of Texas MD Anderson Cancer Center, University of California San Francisco, University of Minnesota Medical School Duluth and University of South Florida Moffitt Cancer Center.

3.1. Randomization

Subjects will be randomized in equal number to the seven conditions using block randomization with blocks of seven and fourteen. Randomization will be stratified by study center. Further details regarding randomization can be found in the Randomization Description.

3.2. Sample Size

Our primary analysis will focus on the number of cigarettes per day at six weeks. Our study is powered to detect a significant difference between conditions (2) – (5) and condition (1). Power analyses are based on two-sided, two-sample t-tests at the 1.25% level to control for multiple comparisons of VLNC cigarettes to normal nicotine content (NNC) controls (4 nicotine yields; 4x1.25 = 5% level). With 96 completers per group (based on 20% attrition over the 6 week period), we will achieve 80% (90%) power to detect an effect size of 0.486 (0.550) which is less than the effect of VLNC vs. NNC cigarettes observed by Hatsukami et al. (2010) for cigarettes per day (CPD; 0.991), cotinine (2.096), FTND (0.603), and withdrawal (0.512).

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.

4.2. Definition of Sub-Group Population in Different Analyses

We intend to complete pre-planned subgroup analyses by sex, race (white vs. black) and menthol status. Subgroup analysis will allow us to evaluate the consistency of the effect of VLNC cigarettes across important subgroups. This information will be crucial for evaluating the effects of cigarettes varying in nicotine content.

5. Trial Endpoints

5.1. Primary Endpoints

- Number of cigarettes smoked per day: study cigarettes, combined study and non-study cigarettes

5.2. Secondary Endpoints

- Measures of compliance: non-study cigarette use, drop-out rate
- Measures of discomfort/dysfunction: MNWS, PANAS, QSU, PSS-4, CESD
- Measures of other health-related behaviors: breathe alcohol, urine drug screen, TLFB-marijuana use, Alcohol Use Questionnaire, Drug Use Questionnaire, DAST, SMAST, weight
- Measures of nicotine/tobacco dependence: FTND, WISDM
- Measures of tobacco exposure: CO, total nicotine equivalents, NNN, NNAL, minor alkaloids

- Measures of intention to quit: Stages of Change, Contemplation Ladder
- Measures of compensatory smoking: puff topography, filter analysis
- Measures of other tobacco use: TLFB-other tobacco
- Measures of cigarette characteristics: CES, Cigarette Purchase Task
- Measures of cognitive function: N-back, 2-Letter search, CPT, arithmetic task
- Measures of cardiovascular function: heart rate, blood pressure, urine 11-dehydroTXB2
- Measures of perceived risk: Perceived Health Risk questionnaire

5.3. Exploratory Endpoint

- Self-reported abstinence

5.4. Safety Endpoints

- Adverse Events (AEs)
- Serious adverse events (SAEs)
- Health Changes Questionnaire
- Respiratory Health Questionnaire

6. Statistical Analysis

6.1. General Approach

The primary objective of this trial is to evaluate the relationship between cigarettes varying in nicotine content and number of cigarettes smoked per day. The secondary objective is to evaluate the relationship between cigarettes varying in nicotine content and nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. In general, each endpoint will be summarized by condition and formal hypothesis testing will be completed to compare across conditions. For the primary endpoint, hypothesis testing will focus on comparing conditions (2) – (5) to the primary control condition (1), while hypothesis testing for the secondary endpoints will include comparisons between conditions (2) – (5). Comparisons with conditions (6) and (7) will not be the primary focus of this study but will provide additional information to help us fully understand the effect of VLNC cigarettes.

Statistical analyses will be performed using SAS (version 9.2 or 9.3) or R. All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below.

All statistical tests will be two-tailed and p-values less than 0.05 will be considered significant. For the primary endpoint, our interest lies in comparing experimental conditions (2) – (5) to control condition (1). A Bonferonni multiple-comparisons adjustment will be used to account for multiple comparisons of the primary endpoint. Therefore, p-values less than 0.05/4 = 0.0125 will be considered significant for all analyses of the primary endpoint. No a priori adjustment for multiple comparisons for analyses of the secondary and exploratory endpoints will be specified since the number of comparisons of interest will be partially determined by the

findings for the primary endpoint; however, the approach will be to use Bonferonni-type adjustments that consider the total number of contrasts made.

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, sex and race), smoking characteristics (cigarettes per day and menthol status) and characteristics of nicotine exposure and dependence (total score on FTND and nicotine metabolic ratio). Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA. Categorical covariates will be summarized by frequencies and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate.

6.3. Primary Endpoint Analysis

The primary endpoint is cigarettes per day at six weeks. The number of cigarettes per day will be recorded daily throughout the study by the Interactive Voice Response (IVR) system. The number of cigarettes per day will be summarized for analysis by averaging the number of cigarettes per day from the previous visit to the current visit. For example, the number of cigarettes per day at week one will be the average number of cigarettes smoked per day between the baseline visit and the week one study visit. Therefore, the number of cigarettes per day at six weeks will be the average number of cigarettes per day between the week five and week six clinic visit.

6.3.1. Primary Analysis

The number of cigarettes per day at week six will be summarized by group using the mean and standard deviation. Mean number of cigarettes per day at six weeks will be compared across groups using linear regression. We expect groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis for all endpoints will only adjust for number of cigarettes per day at baseline (for precision). Pairwise comparisons between experimental conditions found to be significantly different from the control condition will be completed if significant differences from the control condition are observed. A Bonferonni multiple comparison adjustment will be used for the pairwise comparisons with the specific alpha level determined by the number of pairwise comparisons completed.

6.3.2. Secondary Analysis

As a secondary analysis, we will complete an adjusted analysis to account for any baseline imbalances. This analysis will adjust for 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.

Finally, we will analyze the number of cigarettes per day from all visits 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, number of cigarettes per day at baseline and study center. A random intercept for each subject will also be included in the model to account for within subject correlation. This analysis will provide useful information about the change in the number of cigarettes smoked per day over time.

6.4. Secondary Endpoint Analysis

Secondary endpoints will be summarized by treatment group using the mean and standard deviation. Secondary endpoints will be compared using linear regression, adjusting only for the baseline value for precision. Pairwise comparisons will be selected based on the findings for the primary endpoint and the specific research question with Bonferroni-type adjustments for multiple comparisons. In addition, we will also complete an adjusted analysis using linear regression adjusting for age, sex 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. Finally, a linear mixed model analysis will also be completed for secondary endpoints measured at multiple visits.

We expect that biomarkers of exposure, as possibly other secondary endpoints, will be skewed and will be log-transformed for analysis. These variables will be summarized using the geometric mean and differences between groups will be summarized by ratios of geometric means.

6.5. Exploratory Endpoint Analysis

Our exploratory endpoint, self-reported abstinence, will be summarized by treatment group using frequencies and percentages and compared by the chi-squared test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with self-reported abstinence.

6.6. Subgroup Analyses

Subgroup analyses will play an important role in understanding the effects of cigarettes varying in nicotine content. Subgroup analyses will follow the same approach described in Sections 6.2 – 6.4 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 the relationship between cigarettes varying in nicotine content and the study endpoints.

The primary subgroups of interest are defined by sex (men vs. women), race (white vs. black) and menthol status (non-menthol vs. menthol). Given equal recruitment of males and females, we will also be able to detect an effect size of 0.693 (0.785) for analyses focused on a single sex (n=48/group). This will provide us with adequate power to test our primary endpoint (cigarettes per day) within a single sex but limited power for our secondary endpoints.

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 safety outcomes will be analyzed as secondary outcomes. For example, two potential risks to study participants identified in the Data Safety and Monitoring Plan (DSMP), nicotine withdrawal and compensatory smoking, will be evaluated by the secondary endpoints of withdrawal symptoms and biomarkers of exposure.

AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared across treatment groups. We expect SAEs to be extremely rare in this trial and, therefore, no formal statistical comparison of the rate of AEs and SAEs across treatment groups is planned for this trial.

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. That said, 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.

We will compare subjects with and without missing data in order to identify baseline covariates associated with missing data. We will then complete a sensitivity analysis of primary and secondary endpoints using multiple imputation where missing values are imputed using regression models developed from baseline covariates (Little and Rubin, 2002). Finally, we will complete an additional sensitivity analysis where missing data are replaced by baseline values. This will serve as a “worse-case scenario” as treatment group will be, on average, balanced at baseline. These two analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

6.9. Interim Analyses

No formal statistical stopping rule will be used for interim monitoring of the primary or secondary endpoints. The purpose of this study is to understand the relationship between cigarettes varying in nicotine content and the study endpoints. Given the complex and multi-faceted nature of the relationship, it is not feasible to devise a priori rules for stopping early. Therefore, pre-planned interim analyses will only consider endpoints associated with safety and study integrity. This includes cigarettes per day, use of other tobacco products, measures of compliance, AEs/SAEs (including high CO and CESD scores), retention rates, and any other variables requested by the Data Safety Monitoring Board (DSMB).

A report describing the interim results will be created for review by the DSMB at their annual meeting. All results in the DSMB report will be blinded with treatment groups identified by a letter code (A, B, C, etc.). Codes to break the blind can be provided to the DSMB should the need arise to break the blind because unusual patterns are observed in the data.

The statistical analyses described above would require the statistician to be unblinded in order to create a report describing the interim results for the DSMB. Therefore, the statistical analysis for the interim results reported to the DSMB will only include summaries by treatment groups (mean and standard deviation for continuous endpoints, frequencies and percentages for categorical outcomes) and a p-value for an omnibus test of any difference between treatment groups.

The previously described plan for interim analyses relate to pre-planned interim analyses required for the monitoring of safety by the DSMB. It is possible that an ad hoc interim analysis will have to be completed in order to inform CENIC investigators for planning purposes relating to other CENIC studies. In this event, the repeated confidence interval approach (Jennison and Turnbull, 1989; Jennison and Turnbull, 1999) will be used to correct the type-I error rate due to multiple looks at the data. Repeated confidence intervals will be developed using the error spending function proposed by Kim and DeMets (1987):

$$f(t) = \alpha * t^{\rho}$$

with $\rho = 4$. This allows for the flexibility of accommodating an unplanned interim analysis, while protecting the integrity of the study by controlling the type-I error rate. An ad hoc interim analysis that aided CENIC staff in planning other CENIC studies would necessarily be unblinded. Any unblinded interim analysis would be limited to involve only a small number of essential study personal and would not involve any study personal with patient involvement. Furthermore, any unblinded interim analysis would kept confidential from all other study personal in order to protect the integrity of the study.

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Project 1, Study 1:

Investigating the Impact of Nicotine Using Spectrum Cigarettes

Statistical Analysis Plan

2/26/2014

Table of Contents

1. Introduction

This document will serve as the Statistical Analysis Plan for CENIC Project 1, Study 1. This document describes the planned statistical analysis for evaluating the dose-response relationship for very low nicotine content (VLNC) cigarettes. Details for the proposed analysis of the primary, secondary and exploratory endpoints are provided.

2. Trial Objectives

Project 1, Study 1 will evaluate the relationship between nicotine yield of VLNC cigarettes and cigarettes smoked per day, nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. In addition, we will also evaluate product compliance for the various yields of VLNC cigarettes.

3. Trial Design

This is a randomized, multi-center, double-blind study design. 840 subjects will be randomized to one of seven conditions. Participants in each condition will be assigned a cigarette that matches their menthol preference. The groups are as follows:

Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
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2	NRC401	RN-Men	0.26 ± 0.06	9 ± 1.5	0.20 - 0.32
3	NRC300	RN	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
3	NRC301	RN-Men	0.12 ± 0.03	9 ± 1.5	0.09 - 0.15
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4	NRC201	RN-Men	0.07 ± 0.02	9 ± 1.5	0.05 - 0.09
5	NRC102	RN	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04
5	NRC103	RN-Men	0.03 ± 0.01	9 ± 1.5	0.02 - 0.04

6	NRC104	RN-HT	0.04 ± 0.02	13 ± 2	0.02 - 0.06
6	NRC105	RN-HT-Men	0.04 ± 0.02	13 ± 2	0.02 - 0.06

Non-Spectrum Cigarettes to be Used in ***Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes***

Condition	TPMF Code	Type*	Specifications Nicotine Yield	Specifications Tar Yield	Specification Range for Nicotine Yield
7	N/A	USUAL BRAND	N/A	N/A	N/A
7	N/A	USUAL BRAND - Men	N/A	N/A	N/A

*Legend:	
RN-HV	Reduced Nicotine-High Ventilation
RN-HV-Men	Reduced Nicotine-High Ventilation Menthol
RN	Reduced Nicotine
RN-Men	Reduced Nicotine-Menthol
RN-HT	Reduced Nicotine-High Tar
RN-HT-Men	Reduced Nicotine-High Tar-Menthol
CN	Conventional Nicotine
CN-Men	Conventional Nicotine-Menthol
CN-HT-Men	Conventional Nicotine-High Tar-Menthol
LTNR	Low Tar/Nicotine Ratio
LTNR-Men	Low Tar/Nicotine Ratio-Menthol

Condition (1) will serve as the primary control condition, with conditions (2) – (5) serving as the primary experimental conditions. Condition (6) is an exploratory condition to determine the impact of varying the tar content on the acceptability of VLNC cigarettes, while condition (7) will serve as a second control condition to determine the impact of switching brands on the number of cigarettes smoked per day.

Subjects will be enrolled at 10 study centers (84 subjects per center): University of Pittsburgh, University of Minnesota Masonic Cancer Center, Johns Hopkins University, Brown University,

University of Pennsylvania, Duke University, University of Texas MD Anderson Cancer Center, University of California San Francisco, University of Minnesota Medical School Duluth and University of South Florida Moffitt Cancer Center.

3.1. Randomization

Subjects will be randomized in equal number to the seven conditions using block randomization with blocks of seven and fourteen. Randomization will be stratified by study center. Further details regarding randomization can be found in the Randomization Description.

3.2. Sample Size

Our primary analysis will focus on the number of cigarettes per day at six weeks. Our study is powered to detect a significant difference between conditions (2) – (5) and condition (1). Power analyses are based on two-sided, two-sample t-tests at the 1.25% level to control for multiple comparisons of VLNC cigarettes to normal nicotine content (NNC) controls (4 nicotine yields; 4x1.25 = 5% level). With 96 completers per group (based on 20% attrition over the 6 week period), we will achieve 80% (90%) power to detect an effect size of 0.486 (0.550) which is less than the effect of VLNC vs. NNC cigarettes observed by Hatsukami et al. (2010) for cigarettes per day (CPD; 0.991), cotinine (2.096), FTND (0.603), and withdrawal (0.512).

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.

4.2. Definition of Sub-Group Population in Different Analyses

We intend to complete pre-planned subgroup analyses by sex, race (white vs. black) and menthol status. Subgroup analysis will allow us to evaluate the consistency of the effect of VLNC cigarettes across important subgroups. This information will be crucial for evaluating the effects of cigarettes varying in nicotine content.

5. Trial Endpoints

5.1. Primary Endpoints

- Number of cigarettes smoked per day: study cigarettes, combined study and non-study cigarettes

5.2. Secondary Endpoints

- Measures of compliance: non-study cigarette use, drop-out rate
- Measures of discomfort/dysfunction: MNWS, PANAS, QSU, PSS-4, CESD

- Measures of other health-related behaviors: breathe alcohol, urine drug screen, TLFB-marijuana use, Alcohol Use Questionnaire, Drug Use Questionnaire, DAST, SMAST, weight
- Measures of nicotine/tobacco dependence: FTND, WISDM
- Measures of tobacco exposure: CO, total nicotine equivalents, NNN, NNAL, minor alkaloids
- Measures of intention to quit: Stages of Change, Contemplation Ladder
- Measures of compensatory smoking: puff topography, filter analysis
- Measures of other tobacco use: TLFB-other tobacco
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- Measures of cognitive function: N-back, 2-Letter search, CPT, arithmetic task
- Measures of cardiovascular function: heart rate, blood pressure, urine 11-dehydroTXB2
- Measures of perceived risk: Perceived Health Risk questionnaire

5.3. Exploratory Endpoint

- Self-reported abstinence

5.4. Safety Endpoints

- Adverse Events (AEs)
- Serious adverse events (SAEs)
- Health Changes Questionnaire
- Respiratory Health Questionnaire

6. Statistical Analysis

6.1. General Approach

The primary objective of this trial is to evaluate the relationship between cigarettes varying in nicotine content and number of cigarettes smoked per day. The secondary objective is to evaluate the relationship between cigarettes varying in nicotine content and nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. In general, each endpoint will be summarized by condition and formal hypothesis testing will be completed to compare across conditions. For the primary endpoint, hypothesis testing will focus on comparing conditions (2) – (5) to the primary control condition (1), while hypothesis testing for the secondary endpoints will include comparisons between conditions (2) – (5). Comparisons with conditions (6) and (7) will not be the primary focus of this study but will provide additional information to help us fully understand the effect of VLNC cigarettes.

Statistical analyses will be performed using SAS (version 9.2 or 9.3) or R. All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below.

All statistical tests will be two-tailed and p-values less than 0.05 will be considered significant. For the primary endpoint, our interest lies in comparing experimental conditions (2) – (5) to control condition (1). A Bonferonni multiple-comparisons adjustment will be used to account for multiple comparisons of the primary endpoint. Therefore, p-values less than 0.05/4 = 0.0125 will be considered significant for all analyses of the primary endpoint. No a priori adjustment for multiple comparisons for analyses of the secondary and exploratory endpoints will be specified since the number of comparisons of interest will be partially determined by the findings for the primary endpoint; however, the approach will be to use Bonferonni-type adjustments that consider the total number of contrasts made.

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, sex and race), smoking characteristics (cigarettes per day and menthol status) and characteristics of nicotine exposure and dependence (total score on FTND and nicotine metabolic ratio). Continuous covariates will be summarized by the mean, standard deviation, median and range and compared by one-way ANOVA. Categorical covariates will be summarized by frequencies and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate.

6.3. Primary Endpoint Analysis

The primary endpoint is cigarettes per day at six weeks. The number of cigarettes per day will be recorded daily throughout the study by the Interactive Voice Response (IVR) system. The number of cigarettes per day will be summarized for analysis by averaging the number of cigarettes per day from the previous visit to the current visit. For example, the number of cigarettes per day at week one will be the average number of cigarettes smoked per day between the baseline visit and the week one study visit. Therefore, the number of cigarettes per day at six weeks will be the average number of cigarettes per day between the week five and week six clinic visit.

6.3.1. Primary Analysis

The number of cigarettes per day at week six will be summarized by group using the mean and standard deviation. Mean number of cigarettes per day at six weeks will be compared across groups using linear regression. We expect groups to, on average, be balanced for important baseline characteristics due to randomization. Therefore, our primary analysis for all endpoints will only adjust for number of cigarettes per day at baseline (for precision). Pairwise comparisons between experimental conditions found to be significantly different from the control condition will be completed if significant

differences from the control condition are observed. A Bonferonni multiple comparison adjustment will be used for the pairwise comparisons with the specific alpha level determined by the number of pairwise comparisons completed.

6.3.2. Secondary Analysis

As a secondary analysis, we will complete an adjusted analysis to account for any baseline imbalances. This analysis will adjust for 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.

Finally, we will analyze the number of cigarettes per day from all visits 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, number of cigarettes per day at baseline and study center. A random intercept for each subject will also be included in the model to account for within subject correlation. This analysis will provide useful information about the change in the number of cigarettes smoked per day over time.

6.4. Secondary Endpoint Analysis

Secondary endpoints will be summarized by treatment group using the mean and standard deviation. Secondary endpoints will be compared using linear regression, adjusting only for the baseline value for precision. Pairwise comparisons will be selected based on the findings for the primary endpoint and the specific research question with Bonferroni-type adjustments for multiple comparisons. In addition, we will also complete an adjusted analysis using linear regression adjusting for age, sex 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. Finally, a linear mixed model analysis will also be completed for secondary endpoints measured at multiple visits.

We expect that biomarkers of exposure, as possibly other secondary endpoints, will be skewed and will be log-transformed for analysis. These variables will be summarized using the geometric mean and differences between groups will be summarized by ratios of geometric means.

6.5. Exploratory Endpoint Analysis

Our exploratory endpoint, self-reported abstinence, will be summarized by treatment group using frequencies and percentages and compared by the chi-squared test or Fisher's exact test, as appropriate. A secondary analysis using logistic regression will be completed to identify factors associated with self-reported abstinence.

6.6. Subgroup Analyses

Subgroup analyses will play an important role in understanding the effects of cigarettes varying in nicotine content. Subgroup analyses will follow the same approach described in Sections 6.2 – 6.4 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 the relationship between cigarettes varying in nicotine content and the study endpoints.

The primary subgroups of interest are defined by sex (men vs. women), race (white vs. black) and menthol status (non-menthol vs. menthol). Given equal recruitment of males and females, we will also be able to detect an effect size of 0.693 (0.785) for analyses focused on a single sex (n=48/group). This will provide us with adequate power to test our primary endpoint (cigarettes per day) within a single sex but limited power for our secondary endpoints.

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 safety outcomes will be analyzed as secondary outcomes. For example, two potential risks to study participants identified in the Data Safety and Monitoring Plan (DSMP), nicotine withdrawal and compensatory smoking, will be evaluated by the secondary endpoints of withdrawal symptoms and biomarkers of exposure.

AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared across treatment groups. We expect SAEs to be extremely rare in this trial and, therefore, no formal statistical comparison of the rate of AEs and SAEs across treatment groups is planned for this trial.

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. That said, 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.

We will compare subjects with and without missing data in order to identify baseline covariates associated with missing data. We will then complete a sensitivity analysis of primary and secondary endpoints using multiple imputation where missing values are imputed using regression models developed from baseline covariates (Little and Rubin, 2002). Finally, we will complete an additional sensitivity analysis where missing data are replaced by baseline values. This will serve as a "worse-case scenario" as treatment group will be, on average, balanced at baseline. These two analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

6.9. Interim Analyses

No formal statistical stopping rule will be used for interim monitoring of the primary or secondary endpoints. The purpose of this study is to understand the relationship between cigarettes varying in nicotine content and the study endpoints. Given the complex and multi-faceted nature of the relationship, it is not feasible to devise a priori rules for stopping early. Therefore, pre-planned interim analyses will only consider endpoints associated with safety and study integrity. This includes cigarettes per day, use of other tobacco products, measures of compliance, AEs/SAEs (including high CO and CESD scores), retention rates, and any other variables requested by the Data Safety Monitoring Board (DSMB).

A report describing the interim results will be created for review by the DSMB at their annual meeting. All results in the DSMB report will be blinded with treatment groups identified by a letter code (A, B, C, etc.). Codes to break the blind can be provided to the DSMB should the need arise to break the blind because unusual patterns are observed in the data.

The statistical analyses described above would require the statistician to be unblinded in order to create a report describing the interim results for the DSMB. Therefore, the statistical analysis for the interim results reported to the DSMB will only include summaries by treatment groups (mean and standard deviation for continuous endpoints, frequencies and percentages for categorical outcomes) and a p-value for an omnibus test of any difference between treatment groups.

A separate interim analysis will be completed to inform investigators for planning purposes relating to future studies. This analysis will necessarily be unblinded. Only data from participants who have completed their abstinence assessment session as of February 28, 2014 will be included in the interim analysis dataset. In addition, interim data will be used for an analysis relating to compensatory smoking. These results may be reported and may include cigarettes per day as a secondary analysis. A separate plan for this analysis will developed in a separate document. These analyses will not impact the planned study sample size or be used to stop the study early. For this reason, a formal group sequential approach for controlling the type-I error rate will not be used. In a standard group sequential clinical trial, several pre-planned interim analyses are completed and the study terminated when a significant result is

detected. This will inflate the type-I error rate and group sequential methods are needed to control the type-I error rate. In our case, we will enroll the original sample size and present the final study results regardless of the results of the interim analysis. This will not inflate the type-I error rate of our study and, therefore, formal approaches to controlling the type-I error rate are not needed.

References

Hatsukami, D. K., Kotlyar, M., Hertsgaards, L. A., Zhang, Y., Carmella, S. G., Jensen, J. S., et al. (2010). Reduced nicotine content cigarettes: Effects on toxicant exposure, dependence and cessation. *Addiction, 105,* 343-355. doi: 10.1111/j.1360-0443.2009.02780.x.

Little, R.J.A., Rubin, D.B. (2002). Statistical Analysis with Missing Data, Second Edition, John Wiley & Sons, Inc., Hoboken, New Jersey.

Verbeke, G., Molenberghs, G., (2000). Linear Mixed Models for Longitudinal Data, Springer, New York, NY.

P1S1 Statistical Analysis Plan Amendments

Only update: 2/26/15

- Final SAP section 6.9. Interim Analyses
    - The change clarified the approach to an interim analysis that was conducted in March, 2015 for the purpose of selecting the products to use in subsequent studies and as part of an analysis of several datasets related to compensatory smoking. Because the determination was made that the interim analysis would not impact the planned study sample size or be used to determine if the study should be stopped, a formal group sequential approach for controlling the type 1 error rate was determined to be unnecessary. We sought the input of experts in this area (Dr. Jim Neaton) and the DSMB including Dr. Linda Collins who agreed with this determination and approved the change to the SAP.

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATORS:

RESEARCH STAFF:

SOURCE OF SUPPORT: National Institute on Drug Abuse

Why is this research being done?

You are invited to participate in a [INSERT UNIVERSITY NAME HERE] research study investigating the impact of different nicotine levels in cigarettes. This research may help inform the Food and Drug Administration how best to regulate tobacco products in the future with the goal of improving public health. This is not a treatment program for smoking.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you are a smoker who is not currently seeking treatment for quitting smoking. People invited to participate in this study must be at least 18 years old, healthy and, if female, cannot be pregnant or breastfeeding. A total of 300 individuals may be asked to participate.

What procedures will be performed for research purposes?

Screening Procedures

Signing this consent form does not mean that you will be able to take part in this study. You will undergo screening tests to help the study team determine if you are eligible to take part in this study. If you agree to participate in the screening, you will be asked to do several things.

First, we will ask you to blow into a small machine that will tell us whether you have been drinking alcohol recently. If so, we will stop the interview and ask you to come back at another time if you would like to try again. If you come back the second time and test positive for alcohol again, you will not be able to participate in the study. We will also ask for a sample of your urine that will tell us whether you have recently used some illegal drugs. If the test indicates that you have used cocaine, opiates, benzodiazepines, barbiturates, amphetamines, methamphetamines, methadone or PCP we will stop the interview and ask you to come back at another time if you would like to try again. If you come back the second time and test positive for drugs again, you will not be able to participate in the study. Please let us know if you have a valid prescription for the medication that may have caused you to fail the drug test. If you are a woman, we will also do a urine pregnancy test. If the tests show that you are pregnant, you will not be able to be in the study. After that, you will be asked to blow into another small machine that will tell us how much you have been smoking recently. If the test indicates you are not a regular smoker, we may ask for a urine sample to test for nicotine. If this test also indicates you are not a regular smoker, you will be dismissed from the study. Then, we will ask you questions about

your medical history, medication use, current and past smoking behavior, and how you feel. After we finish with these activities, we will make an initial decision about your eligibility and the research assistant will schedule your first baseline visit if he/she thinks you will most likely be eligible. We will give your medical history to our licensed medical professional, [INSERT NAME HERE], who will have the final decision as to whether you are able to participate in the study. If the licensed medical professional is available, he/she may meet with you to discuss your medical history in order to better determine your eligibility. In the event he/she does not think it is appropriate for you to participate in the study, the research assistant will contact you prior to the first baseline visit to cancel your appointment. Your participation in this screening interview is voluntary, which means that you can leave at any time if you lose interest or are uncomfortable.

You will receive $25 for completing this screening session, even if you are not eligible for the study or decide that you do not want to participate as long as you pass the drug test, breath alcohol test, and the tests indicating you are a regular smoker. If you do not pass these tests you will be dismissed from the study without payment, unless you have a prescription for the medication that caused you to fail the drug test.

Study Procedures

If after this screening visit, the licensed medical professional determines that you are eligible to participate in the study and you decide to participate in the study, you will be required to come back to the lab for 9 additional visits that will take place over 8 to 12 weeks. The first 2 visits will last 1-2 hours each. In these visits, we will ask you questions related to your health, smoking and mood. We will ask you to do some short tests on the computer that measure attention and decision making. Next, we will show you some computer equipment that measures how you smoke. We will ask you to smoke a cigarette through a mouthpiece that is attached to this equipment. During one visit, we will ask you to provide us with saliva samples while you are in the laboratory as well as bringing a urine sample from home. We will test the urine and saliva samples for levels of nicotine and other chemicals that are in cigarettes. If you are a woman, we will ask you to provide us with a urine sample while you are in the laboratory. We will use this sample to perform a urine pregnancy test. If the tests show that you are pregnant, you will be withdrawn from the study. Prior to one of your visits, we will ask you to keep track of all your cigarette butts for a 24 hour period of time and bring them with you to the lab. Additionally, during another visit a saliva sample will be collected to be stored and used for DNA analysis in the future. The DNA sample will be used to determine if there are links between your genes and levels of tobacco constituents, behavior, mood, brain functioning, and the harmful effects of smoking. If you chose not to provide a DNA sample you are still allowed to participate in the study.

We will also teach you how to use an automated (computer) phone system that will call you every day during the study to ask how many cigarettes you smoked that day and, on some occasions, will ask about your mood. This phone system is operated by InterVision Media, a media production company. To be enrolled in this phone system, you will be providing their website with some identifiable information (initials and telephone number only). This identifiable information will be used for website enrollment only; the data analysis group will not have access to it nor will it be shared with anyone outside of the InterVision Media company.

At the end of the second visit, you will be randomly placed into one of seven cigarette groups. The cigarette groups will vary in nicotine and/or tar yield. You will not be aware of the nicotine and/or tar yield of your assigned cigarette until after the entire study has been completed. It is possible you will be smoking cigarettes that contain less nicotine than what is found in most brands. Some cigarettes may

have higher tar yields than others. You could also be assigned to a cigarette that has levels of nicotine and tar that are similar to cigarettes available in stores. One group will be assigned to their usual brand.

You will smoke your assigned cigarette for the remainder of the study. We will give you more than enough cigarettes to replace the amount that you usually smoke each week. We will ask you to try to smoke only the cigarettes we give you. We will work with you to achieve this goal as it is very important for the study. However, it is important that you inform us of any non-study cigarettes, tobacco or nicotine products that you use. Additionally, we need to keep track of the cigarettes we give to you. Therefore, we would like you to keep all of your empty cigarette packs and unused cigarettes and bring them back to the lab each week.

Although this study is not a treatment program, if you choose to reduce the amount you smoke or stop smoking entirely you are still able to participate in the study. If you decide to quit, we will provide you with information about stopping smoking and referrals to local treatment programs. If you decide to quit, you can choose whether you want to take home your assigned study cigarettes. Having cigarettes in your possession during your quit attempt could make it difficult for you to refrain from smoking.

Once you are assigned a cigarette, you will be visiting the laboratory every week for the remainder of the study. At each visit, we will ask you questions about your smoking, mood, craving for cigarettes, and about the cigarettes that you have been using. We will also get a breath sample to measure how much you have been smoking. During some visits, we will ask you to repeat the computer tests of attention and decision making and ask you to smoke a cigarette through the device that measures how you smoke. We will collect the cigarette butts to measure the differences in the amount of nicotine and tar people take into their bodies when smoking. Also during some visits, we will ask you to provide us with a urine sample while you are in the laboratory as well as bringing a urine sample from home. We will test the urine samples for levels of nicotine and other chemicals that are in cigarettes. Additionally, we will test the urine samples for illicit drug use. Results of these drug tests will NOT affect your participation in the study. We will also run an additional urine pregnancy test on female participants of child-bearing potential. Prior to two of your visits, we will ask you to keep track of all your cigarette butts for a 24 hour period of time and bring them with you to the lab. At each visit, we will provide you with your assigned cigarettes as needed, and we will ask you whether you have had any problems using the new cigarettes and whether you have been using any other types of cigarettes or nicotine products.

At the end of the study, you will return to the laboratory for one additional visit which is an abstinence session. We will ask you to not smoke from the time you leave the laboratory the day before until you return for the abstinence session. During the visit we will be asking you questions about your craving and mood and repeating the tests that measure attention and decision making.

Due to your participation in this study, including the use of the experimental cigarettes and the requirement to be abstinent for 24 hours prior to the last session, you may find it easier to quit smoking completely. Therefore, after you complete the study we will talk to you about the benefits of remaining smoke-free and will provide you with information about quitting smoking.

One month after the last study visit, we will call you to ask about your smoking behavior since the study ended.

Compensation

The total amount of money that you could earn for this study is $835. This includes $25 for the screening session, $25 for each of the five shorter sessions, $75 for each of the three longer sessions,

$90 for the abstinence session, a $200 bonus for completing all ten sessions and being on time for your appointments, up to $160 for compliance with the daily telephone calls and $10 for completing the study follow-up phone call. If you start but do not finish the study, you will be compensated for the sessions that you do complete at the same rate.

Data Analysis

This study is taking place at ten locations across the country (University of Pittsburgh, Brown University, Johns Hopkins University, University of Minnesota Masonic Cancer Center, University of Pennsylvania, University of California San Francisco, University of Texas MD Anderson Cancer Center, University of Minnesota Medical School Duluth University of South Florida Moffitt Cancer Center, and Duke University). Identifying information such as, your name, address, phone number, etc. will **NOT** be sent to the University of Minnesota Masonic Cancer Center.

Description of foreseeable risks or discomforts:

1) **Survey Questionnaires:** This interview will include questions about your medical history, drug and alcohol use, and questionnaires about your mood. Answering these personal questions could make you uncomfortable.
2) **Breach of Confidentiality:** The only risk of this interview is your loss of privacy if other people find out the results.
3) **Drug Testing:** A breach of confidentiality could occur, thus others could learn of your drug use. We test for marijuana, cocaine, PCP, opiates, methamphetamines, amphetamines, barbiturates, benzodiazepines and methadone.
4) **Obtaining Blood Pressure and Heart Rate:** The blood pressure cuff may cause minimal discomfort. In obtaining your blood pressure and heart rate we may find that you have an abnormal blood pressure and/or heart rate.
5) **Smoking Cigarettes:** All cigarettes are detrimental to a person's health and can lead significant medical problems including:
    a. Cardiovascular Diseases: Coronary heart disease, heart attack, stroke, peripheral vascular disease, reduced blood circulation, abdominal aortic aneurysm
    b. Respiratory Diseases: Emphysema, bronchitis, and chronic airway obstruction
    c. Cancers: Cancers of the lung, bladder, cervix, esophagus, kidney, larynx, mouth, pancreas, throat, and stomach; leukemia
    d. Other Health Risks Associated with Smoking: Including but not limited to infertility, lower bone density in postmenopausal women, and hip fracture in women
    e. Death

    For more information about the harmful effects of smoking and the benefits of quitting, please visit the Centers for Disease Control and Prevention (CDC) website: http://www.cdc.gov/tobacco/, the National Cancer Institute's website: www.smokefree.gov or call the National Cancer Institute's Smoking Quitline: 1-877-44U-QUIT.
6) **Smoking Study Cigarettes:** In addition to the above medical problems, you may experience some minor adverse health effects such as headaches or experience withdrawal symptoms, which are listed below. Due to the altered nicotine levels, there could be a change in your use of cigarettes including the manner in which you inhale the smoke. Smoking the study cigarette does not provide any less risk than your usual brand of cigarette and could pose increased health risks. You may also experience increases in levels of carbon monoxide, a gas from smoke.
7) **Smoking Withdrawal:** You may experience smoking withdrawal symptoms during this study. These symptoms can include anger, irritability, frustration, anxiousness, depressed mood, craving for a cigarette, difficulty concentrating, increased appetite, weight gain, sleep problems,

restlessness, impatience, constipation, dizziness, coughing, nightmares, nausea and sore throat. These feelings can be uncomfortable but typically are of minimal risk.

8) **Returning to Regular Smoking**: It is possible that if you return to smoking your usual brand of cigarette at the end of the study you may experience mild and transient nausea, dizziness, and lightheadedness.
9) **Risk to Fetus**: Smoking during pregnancy can lead to 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 cigarettes consumption could adversely affect psychiatric conditions.

Avoiding Risks to Fetus:

In order to avoid the previously mentioned risks to a fetus, it is important that you are not pregnant during this study. Avoiding sexual activity is the only certain method to prevent pregnancy. However, if you choose to be sexually active, you 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. If you choose to be sexually active during this study, pregnancy could still result even with the use of these birth control methods. Female participants with child-bearing potential will be tested for pregnancy at the screening visit, before randomization during the Baseline 2 visit and at the Week 6 visit. If you become pregnant during the study, you will be withdrawn from the study. Approximately 30 days after being withdrawn or having a positive pregnancy test at the Week 6 visit, you will receive a call from study staff who will confirm your due date. The site's licensed medical professional will follow-up with you after delivery to ask questions about the baby's health

Expected benefits of study:

You will not benefit from taking part in this study. The information that we get from the study may ultimately help the Food and Drug Administration decide how best to regulate tobacco products with the goal of improving public health.

Other treatment(s) available:

We are not offering treatment for smoking in this study. If you are seeking treatment for smoking, please let us know and we will help you to find a treatment program. Information about quitting smoking can be found by visiting the National Cancer Institute's website: www.smokefree.gov or by calling their national Smoking Quitline: 1-877-44U-QUIT.

Use of research results:

The information that you give us in this study will be confidential. We will talk about and publish the results of this study, but we will never identify you by name. We will keep your records locked in a secure location. However, if we think you intend to seriously harm yourself or someone else, or if there is reason to believe that you have committed child or elder abuse or neglect, that information will be shared with the proper authorities.

Your rights:
You are free to withdraw from the study at any time. Your refusal to participate or withdrawal will involve no penalty or loss of rights to which you are otherwise entitled.

The Principal Investigator also reserves the right to remove you from the study at any time.

Who will pay if I am injured as a result of taking part in this study?
If you believe that the research procedures have resulted in an injury to you, immediately contact the Principal Investigator who is listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the [INSERT HOSPITAL NAME HERE]. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the [INSERT UNIVERSITY NAME HERE] Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives of the sponsor of this research study, the National Institute on Drug Abuse, may review and/or obtain identifiable information related to your participation in this research study for the purpose of monitoring the accuracy and completeness of the research data and for performing required scientific analyses of the research data. While the study sponsor understands the importance of maintaining the confidentiality of your identifiable research information, the [INSERT UNIVERSITY NAME HERE] cannot guarantee the confidentiality of this information after it has been obtained by the study sponsor.

Additionally, authorized representatives from any federal, state or local governmental agency that regulates the study may also have access to your identifiable information. Agencies include the Food and Drug Administration (FDA), the U.S. Department of Health and Human Services (DHHS) and Office for Human Research Protections (OHRP).

Employees at InterVision Media will have access to your initials and telephone number. They will not be provided with any other identifiable information such as your full name, address or social security number.

Genetic Information Nondiscrimination Act (GINA)
A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility of premiums.
- Employers with 15 or more employees may not use your genetic information from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies, group health plans and employers as outlined above must follow this law. Please note that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

As noted above, all data we collect from you, including you genetic data, will be kept separate from information that can be used to identify you. Furthermore, we will use the Certificate of Confidentiality to resist demands for information that would identify you.

Certificate of Confidentiality

To help protect your privacy, the researchers have received a Certificate of Confidentiality from the National Institutes of Health. With this certificate, the researchers cannot be forced to disclose the information that may identify you, 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 you, 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).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself and your involvement in the research. If an insurer, employer or other person obtains your 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 your consent, information that would identify you 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.

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VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, [INSERT UNIVERSITY NAME HERE] [INSERT TELEPHONE NUMBER HERE].

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Name (Print) Date

_____

Participant's Signature

DNA Saliva Sample:

Provide a saliva sample that will be stored for future DNA analysis

☐ I agree _____ **OR** ☐ I disagree _____

Initials Initials

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual, and I have discussed the potential benefits and possible risks of study participation. Any questions the individual has about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date