Reduced Nicotine in Cigarettes in a Marketplace with Alternative Nicotine Systems

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CENIC II PROJECT 1

Impact of Very Low Nicotine Content Cigarettes in a Complex Marketplace*

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Revision History

Revision #	Version Date	Summary Of Changes	Consent Changes
1	7/21/17	Original protocol to CPRC & IRB	Original 21JUL2017
2	4/4/18	Minor corrections to grammar/spelling, clarifications of terms and procedures; update of study products and point exchange procedures; update of Statistical Analysis Plan.	08FEB2018
3	9/27/18	Minor corrections or clarifications Eligibility: CO≥10 ppm or cotinine (nicotine) strip indicating regular smoking Removed 2nd saliva sample for genotyping Revised Risk: E-liquid exposure Baseline Point Calculations: Includes other tobacco/nicotine Added GAD-7 questionnaire Removed buccal cell collection Bonus ($300) will be contingent on avoiding non-study product use; or $100 for accurate self-report of slips At unanticipated visits for additional study product, CO will be done if CPD is >50% above baseline	27SEPT2018
4	10/16/19	Change of N to 500 Addition of 3 sites Addition of tobacco pouches to Marketplace Addition of risk of e-cigarette for pulmonary disease and seizures Exclusion criteria of suicide attempt in last 5 years has been modified Revised statistical analysis	18NOV2019
5	1/17/20	Increase age to 21 for eligibility	None
The COVID-19 pandemic halted subject participation in March 2020. Recruitment for the study resumed in September 2020 following an amendment adapting the in-person visits to telehealth with a curbside component for dropping off biological samples and dispensing the study products. As a result, the first 178 subjects were randomized under the original procedures and subsequent subjects were randomized under the telehealth-curbside amendment procedures. Those procedures are outlined in the COVID-19 AMENDMENT_ Telehealth-Curbside_Amenedment 1 and 2_07.01.20 and 04.08.21 (protocol and consent); document incorporated into final protocol.			
6	05/04/22	Change of N to approximately 425-450 Comments regarding the COVID-19 pandemic amendment for telehealth visits (discontinued BrAC, toxicology; first morning void used for pregnancy screen and saliva sample for compliance testing rather than spot urines) Removed statement that study cell phones provided Updated Statistical Analysis Plan and monitoring plan	None
7	11/23/22	Revised the Statistical Analysis Plan; updated N to 400-450; minor edits or clarifications	None

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Abbreviations

AE: **Adverse Events**: any untoward medical occurrence in a subject administered a study product and which does not necessarily have a causal relationship with this treatment.

ANDS: **Alternative Nicotine Delivery Systems:** products such as e-cigarettes or other vaping devices, smokeless tobacco, medicinal nicotine replacement.

BrAC: **Breath Alcohol Concentration**: measured by an expired breath sample is most commonly used as a metric of alcohol exposure.

BDI-II: **Beck Depression Inventory, 2nd Edition**: a 21-question multiple-choice self-report inventory, used for measuring the severity of depression.

BP: **Blood Pressure:** is one of the principal vital signs.

CENIC: **Center for the Evaluation of Nicotine in Cigarettes:** the short title selected for this program grant that would be easily recognized.

CES: **Cigarette Evaluation Scale:** a 12-item questionnaire that assesses the degree to which smokers experience the reinforcing effects of smoking.

CES-D: **Center for Epidemiological Studies Depression Scale:** is a self-report measure of depression severity.

CO: **Carbon Monoxide:** exhaled breath carbon monoxide level reflects the level of carboxyhemoglobin (HbCO) in blood.

COPD: **Chronic Obstructive Pulmonary Disease:** is a type of obstructive lung disease characterized by chronically poor airflow.

CVD: **Cardiovascular Disease:** (also called heart disease) is a class of diseases that involve the heart, the blood vessels (arteries, capillaries, and veins) or both.

DAST-10: **Drug Abuse Screening Test:** is a 10-item, yes/no self-report instrument for clinical screening of drug abuse and treatment evaluation.

DBP: **Diastolic Blood Pressure**

DVT/PE: **Deep Vein Thrombosis / Pulmonary Embolism:** A deep vein thrombosis is a blood clot in the deep veins of the leg. If the thrombus breaks off (embolizes) and flows towards the lungs, it can become a life-threatening pulmonary embolism (PE), a blood clot in the lungs.

ENDS: **Electronic Nicotine Delivery Systems:** Products such as e-cigarette and other vaping devices.

FTND: **Fagerstrom Test for Nicotine Dependence:** is a 6-item standard instrument for assessing the intensity of physical addiction to nicotine and includes an evaluation of cigarette consumption, the compulsion to use, and dependence.

HCG: **Human Chorionic Gonadotropin:** is a hormone produced by the placenta following implantation. The presence of hCG is detected in pregnancy tests.

HR: **Heart Rate:** a measure of the number of heart beats per minute (bpm)

IVR: **Interactive Voice Response:** is a technology that allows a computer to interact with humans through the use of voice and input via keypad.

MNWS: **Minnesota Nicotine Withdrawal Scale**: is a 15-item self-reported scale to evaluate the effects of smoking cessation.

NIAAA: **National Institute on Alcohol Abuse and Alcoholism:** part of the National Institutes of Health that supports and conducts biomedical and behavioral research on the causes, consequences, treatment, and prevention of alcoholism and alcohol-related problems.

NIDA: **National Institute on Drug Abuse:** part of the National Institutes of Health whose mission is to "lead the Nation in bringing the power of science to bear on drug abuse and addiction."

NIH: **National Institute of Health:** an agency of the United States Department of Health and Human Services, it is the primary agency of the United States government responsible for biomedical and health-related research.

NMR: **Nicotine Metabolite Ratio:** is a salivary measure of the ratio of nicotine metabolites, which indicates speed of nicotine metabolism.

NNAL: **4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol:** A tobacco specific nitrosamines, one of the most important groups of carcinogens in tobacco products, formed from nicotine during the curing and processing of tobacco.

NNC: **Normal Nicotine Content**

PATH: **Population Assessment of Tobacco and Health:** a longitudinal study national study looking at tobacco use and health.

PCP: **Phencyclidine:** known colloquially as Angel Dust and many other names, is a recreational dissociative drug.

PI: **Principal Investigator:** is the lead scientist for a particular well-defined research project, such as a laboratory study or clinical trial.

PrimeMD: **Primary Care Evaluation of Mental Disorders:** Patient Health Questionnaire (PHQ –Pfizer©), a multiple-choice self-report inventory that is used as a screening and diagnostic tool for mental health disorders of depression, anxiety, alcohol, eating, and somatoform.

QSU: **Questionnaire of Smoking Urges-Brief:** a 10 item questionnaire to self-report smoking urges and cravings.

REDCap: **Research Electronic Data Capture (web based software):** is a secure, web-based application for building and managing online surveys and databases.

RNC: **Reduced nicotine content:** cigarettes with lower levels of nicotine than normal cigarettes.

RTI: **Research Triangle Institute:** now RTI International, is a nonprofit organization, headquartered in the Research Triangle Park in North Carolina, that provides research and technical services.

SAE: **Serious Adverse Events:** generally, any event which causes death, permanent damage, birth defects, is life-threatening or requires hospitalization is considered an SAE.

SBP: **Systolic Blood Pressure**

SMAST: **Short Michigan Alcohol Screening Test:** a 13 item screening tool which is derived from one of the oldest alcoholism screening tests in identifying dependent drinkers.

TLFB: **Timeline Followback:** is a method that can be used as a clinical and research tool to obtain a variety of quantitative estimates of marijuana, cigarette, and other drug use by asking clients to retrospectively estimate their usage prior to the interview date.

TNE: **Total nicotine equivalents:** a urinary measure of nicotine and its metabolite concentrations.

VLNC: **Very low nicotine content:** cigarettes with much lower levels of nicotine than normal cigarettes (possibly below addictive levels).

WISDM: **Wisconsin Inventory of Smoking Dependence Motives (Brief):** a measure of tobacco dependence.

Study Synopsis

Study Design: Phase III randomized, open label, multi-site study that will examine the impact of very low nicotine content (VLNC) versus normal nicotine content (NNC) cigarettes in a complex tobacco and nicotine product marketplace simulating a real world environment.

Primary Aims: To examine the effects of cigarettes that vary in nicotine content on the number of cigarettes smoked, smoke-free days , extent of uptake of non-combusted alternative nicotine-containing products, and extent of exposure to tobacco toxicants in the context of a marketplace with alternative nicotine –containing products.

*We hypothesize that subjects in VLNC vs. NNC cigarette condition will experience: 1) fewer cigarettes smoked; 2) greater number of smoke-free days.3) greater uptake of alternative nicotine/tobacco products; and 4) less toxicant expos*ure.

Exploratory analysis will examine whether the type (e.g., electronic nicotine delivery systems) and extent of use of alternative products mediates further reductions in cigarettes and toxicant exposure.

Secondary Aim : **Secondary Aim 1:** To examine the characteristics of products alternative to cigarettes that are chosen and to better understand responses to these various products among smokers assigned to VLNC vs. NNC cigarettes.

We hypothesize that across conditions, flavored electronic nicotine delivery systems with higher doses of nicotine will be preferred over other non-combustible products. Moreover, among subjects who try them, non-combusted products will be rated more positively and as providing greater withdrawal relief in the VLNC as compared to NNC conditions.

Secondary Aim 2: To explore variables that predict responses to the marketplace.

We hypothesize that a variety of factors (i.e., dependence severity, age, sex, racial ethnic group, SES) will impact product use patterns (e.g., the amount of non-combusted vs. combusted products used and days smoke-free within NNC and VLNC cigarette conditions).

Population: Cigarette smokers

Study Procedures: Subjects will undergo two weeks of monitoring of usual brand cigarette smoking and then two weeks access to the experimental marketplace that includes their usual brand but no study cigarettes (to adapt smokers to the experimental marketplace). Subjects will then be randomly assigned for a period of 12 weeks to one of two marketplace conditions with access to: 1) VLNC study cigarettes and non-combusted tobacco and nicotine products; or 2) NNC study cigarettes and non-combusted tobacco and nicotine products. Subjects in both conditions will be unblinded and informed that the study cigarettes contain very low nicotine levels or nicotine levels similar to conventional cigarettes currently sold on the market, respectively. The price of cigarettes and other products will be relative to the prices that are currently in the marketplace.

Accrual: **400-450; approximately 200-225 in each condition (VLNC & NNC).**

Enrollment Period: 3/1/2018-9/30/2022

1. Study Objectives and Hypotheses

1.1. Primary Objective

The primary aim of this study is to examine the effects of cigarettes that vary in nicotine content on the number of cigarettes smoked; number of smoke-free days, extent of uptake of alternative non-combusted product use, and extent of tobacco-related toxicant exposure in the context of a marketplace containing alternative non-combusted nicotine-containing products.

- We hypothesize that the very low nicotine content (VLNC) compared to normal nicotine content (NNC) cigarettes will lead to: 1) fewer cigarettes smoked; 2) greater number of smoke-free days; 3) greater number of days of non-combusted nicotine-containing product use; and 4) less exposure to tobacco-related toxicants. .

Exploratory analysis will examine whether the type (e.g., electronic nicotine delivery systems [ENDS]) and extent of use of alternative products mediates further reductions in cigarettes and toxicant exposure. We hypothesize that uptake of ENDS and greater uptake of alternative products will lead to lower cigarette use and toxicant exposure.

1.2. Secondary Objectives

1.2.a Secondary Aim 1: To examine the characteristics of products alternative to cigarettes that are chosen and to better understand responses to these various products among smokers assigned to VLNC vs. NNC cigarettes.

- We hypothesize that across conditions, flavored electronic nicotine delivery systems with higher doses of nicotine will be preferred over other non-combustible products. Moreover, among subjects who try them, non-combusted products will be rated more positively and as providing greater withdrawal relief in the VLNC as compared to NNC conditions.

1.2.b Secondary Aim 2: To explore variables that predict responses to the marketplace.

- We hypothesize that a variety of factors will impact product use patterns (e.g., the amount of non-combusted vs. combusted products used and days smoke-free within NNC and VLNC cigarette conditions).

2. Background

2.1. Significance

Changing the tobacco product. Over 44 million people in the United States smoke cigarettes[1] and about 1.2 billion globally.[2] With about 500,000 deaths per year in the US and 6 million per year world-wide,[2] it is critical to have strong tobacco control policies in place to minimize the casualties from tobacco use.

The Family Smoking Prevention and Tobacco Control Act (FSPTCA), passed in 2009, provides the FDA with the authority to regulate tobacco products. One of the provisions in this legislative act empowers the FDA to assert product standards that would reduce harmful constituents in

tobacco products, including nicotine as long as the nicotine levels are not reduced to zero. A standard that has the potential to profoundly reduce the tobacco-caused mortality and morbidity is establishing maximum levels on nicotine to render cigarettes minimally addictive.[3,4] Such a measure could reduce the transition of novice smokers from experimentation to becoming addicted, help current smokers quit smoking, and reduce the likelihood that former smokers will relapse. Although the proposal to reduce nicotine in cigarettes has been met with some skepticism because of concerns over the potential for compensatory smoking behavior[5] and the emergence of a black market,[6] this policy measure was considered to be technically feasible by the American Medical Association and the British Medical Association,[3] and by tobacco control researchers, policymakers and governmental officials who were convened in a meeting on nicotine regulation.[7] The public health impact of this approach has been estimated to be equivalent to the impact of sanitation efforts in reducing death and disease.[8] Taking into account any mortality increases due to compensatory smoking or the emergence of a black market; the prevalence of smoking was projected to decline to 5%.[9]

2.2. Existing Literature

Reducing nicotine content in cigarettes. Over 20 years ago, a proposal for a nationwide gradual reduction of nicotine levels in cigarettes was described as a means to decrease the development of cigarette dependence.[10] Although researchers and medical organizations supported this approach as a potential policy measure, a number of research questions were raised that needed to be addressed (e.g., the impact of reduced nicotine content [RNC] cigarettes on smoking behavior, use of other tobacco products, toxicant exposure and cessation). To date, several studies have been conducted on this topic. Two studies conducted by Benowitz et al.[11,12] examined gradual nicotine reduction approaches among smokers not seeking cessation treatments. These studies differed in the duration of smoking of each dose of RNC cigarettes: one week versus one month. The results from both of these studies showed minimal compensatory smoking, substantial decreases in cotinine (a metabolite of nicotine) levels, no increase in exposure to other tobacco toxicants, and minimal withdrawal symptoms. Another study conducted by Hatsukami et al.[13] examined the effects of immediately switching to a very low nicotine content (VLNC) cigarette compared to a higher nicotine content cigarette and to nicotine lozenge in smokers interested in quitting smoking. In the VLNC cigarette condition, decreases in smoking, modest withdrawal symptoms after switching from usual brand cigarettes, reduction in dependence, reduction in toxicant exposure and a moderate cessation rate were observed. Finally, Donny et al conducted a large clinical trial in which cigarettes smokers (n=840) were randomly assigned to 1 of 6 doses of nicotine or an additional very low nicotine dose cigarette but with high tar. This study showed that relative to cigarettes with normal amounts of nicotine (15.8 mg/g), cigarettes with 0.4 mg/g nicotine led to reduced smoking and dependence, reduced craving and withdrawal during abstinence and to increased quit attempts.[14] There was no evidence of increased exposure to various tobacco smoke toxicants. Collectively, these studies suggest that reducing nicotine in cigarettes is a highly promising regulatory approach. Currently, we and other investigators are have analyzed data from a recently completed study to determine optimal approaches to reducing levels of nicotine in cigarettes (gradual vs. immediate

reduction to VLNC) and the impact of VLNC cigarettes in vulnerable populations (e.g. individuals with psychiatric diagnosis). To date, no safety issues have been observed in either the immediate vs. gradual reduction approach or in vulnerable populations.

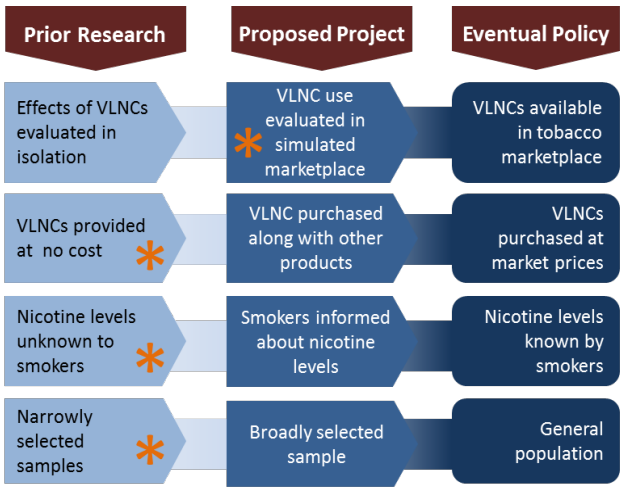


Figure 1. The proposed research will provide novel information on the potential effects of a national nicotine reduction policy by approximating a complex marketplace in a broadly selected sample of smokers. Orange asterisks reflect features of the pilot study in which the effects of VLNCs on use of a wide range of products was evaluated but in which VLNCs were provided free (i.e. not a part of the market), nicotine condition was blinded and the sample was narrowly selected.

2.3. Rationale for conducting current studies.

Prior studies, while experimentally strong, have not fully simulated either the marketplace, or the consumer, in a post-nicotine reduction policy world (Figure1).

With respect to the marketplace, the aforementioned studies were/are primarily focused on examining the impact of RNC cigarettes alone under double-blind conditions; therefore participants were discouraged from using any other tobacco products, cigarettes were provided at no cost, and information about the nicotine content of cigarettes was withheld. These study designs, while able to evaluate the influence of nicotine reduction per se on relevant outcomes, do not adequately simulate a real world marketplace in three major ways. First, with a national nicotine reduction policy, no regular nicotine cigarettes would be available and alternative nicotine-containing products would be widely available. Therefore, the impact of such a policy on the use of other tobacco or nicotine products needs to be considered to fully understand public health effects. Second, providing cigarettes for free is likely to underestimate the degree to which RNC cigarettes might impact smoking behavior. If smokers were required to pay $6 to $10 for a pack of cigarettes that they find relatively non-rewarding, they are likely to smoke fewer cigarettes than they would if these products are provided at no cost.[14] The importance of price on consumption is widely known and is the basis of increasing taxes on cigarettes to drive the prevalence of smoking down.[15] Third, with the implementation of a reduced nicotine policy, the FDA would by necessity provide information to consumers on the reduced nicotine content of cigarettes. The knowledge of nicotine content in cigarettes might influence expectancies or beliefs about the cigarettes, which then might affect smoking uptake or behavior. With respect to simulating the consumer, another limitation of prior studies is the recruitment of a relatively narrow selection of smokers in most studies**.** That is, smokers were recruited only if they were not motivated to quit smoking and used minimal amounts of other tobacco products. In a recent large clinical trial, about 15% were excluded for using other tobacco products (e.g., greater than 9 times per month) and 7% were excluded because they were motivated to quit in the next 30 days, thus omitting almost a quarter of the sample. All these limitations demonstrate the need to conduct a study that includes a more broadly selected sample of smokers and that better approximates the marketplace that is likely to occur if the policy to reduce nicotine in cigarettes is implemented.

Rationale for a Real World Marketplace. The potential effects of RNC cigarettes on use of other tobacco products are illustrated by the findings from several studies. First, even in studies that required smokers to only smoke RNC cigarettes, a significant number smoked usual brand cigarettes, indicating the smokers needed to supplement the reduced nicotine in the study cigarettes with their own cigarettes. For example, in a gradual nicotine reduction study conducted by Benowitz et al.,[12] 10% (N=59) of smokers (not seeking treatment) assigned to RNC cigarettes withdrew during the tapering phase and 21% (N=53) of subjects who completed the tapering phase reported smoking some commercial cigarettes. In Hatsukami et al.,[13] about 30.4% (N=37) of smokers (seeking smoking cessation treatment) who were assigned to 0.6 mg/g nicotine content cigarettes (0.05 mg machine determined nicotine yield) for six weeks used non-assigned tobacco products in the first week and by six weeks, about 5.3% (N=32) reported using such products. These rates are likely to be underestimates because they were not biochemically verified. For example, in the large study conducted by Donny et al.,[14] in a secondary analysis of product non-compliance, about three-fourths of participants using the 0.4 mg/g nicotine content cigarettes (0.04 mg machine determined nicotine yield) were biochemically confirmed to be using some non-study tobacco products based on levels of total nicotine equivalents (TNEs).

In a study conducted by Hatsukami et al.,[16] smokers assigned to VLNC with access to alternative nicotine delivery systems (ANDS) reported greater uptake of these products compared to those assigned to normal nicotine content cigarette; and they also experienced significantly greater reductions in combusted product use, nicotine exposure, cigarette dependence and higher number of quit attempts. Furthermore, the amount of non-combusted ANDS use was significantly, negatively associated with the amount of combusted product use and levels of carcinogen exposure, and positively related to number of abstinence days.

***Rationale for seeking alternative nicotine sources*.** The reasons for seeking alternative nicotine sources among smokers assigned to VLNC cigarettes can be multi-fold. Smokers generally report that VLNC cigarettes are low in satisfaction/liking, enjoyable sensations, and psychological reward compared to NNC cigarettes.[17] Therefore, smokers may be seeking products that have similar reinforcing value as NNC cigarettes. Smokers may also use alternative products in an attempt to reduce any nicotine withdrawal symptoms associated with smoking VLNC cigarettes. In another study conducted by Hatsukami et al.,[18] smokers (seeking smoking cessation treatment) were provided nicotine patch in addition to VLNC (0.6 mg/g nicotine content) cigarettes or VLNC cigarettes alone. This study found that use of the nicotine patch can reduce withdrawal symptoms resulting from switching to VLNC cigarettes, suggesting that other nicotine-containing products may also have these effects. Other factors that might promote uptake and persistent use of alternative products include perceived reduced health risks or use as a means for cessation[19-21] and for those who are assigned VLNC, more positive product expectancies of the alternatives than the VLNC cigarettes. Although studies have been conducted comparing subjective responses to VLNC with NNC cigarettes, to date, no studies have compared subjective responses to non-combusted nicotine-containing products in the context in which cigarettes with different doses of nicotine are available.

***Predictors of use of alternative nicotine/tobacco products*:** In the aforementioned study on the impact of nicotine content on the use of ANDS,[16] predictors of greater use of these alternative products were being male and non-white, reporting higher dependence, and having higher education and income levels (all p's < 0.0001). Some of these results are concordant with results from Donny et al.,[14] in which indicators of dependence (cigarettes per day, urinary total nicotine equivalents) were associated with non-compliance with study cigarette use only, which could be a proxy for when smokers feel the need to seek alternative sources of nicotine. The predictor variables are also consistent with the literature that shows that men are more sensitive to nicotine than women,[22,23] that dependence severity predicts the need for higher nicotine doses,[24] and that use of alternative products, particularly electronic nicotine delivery systems, is greater among those with higher education.[25,26] These results underscore the need to further investigate how different populations of smokers will respond to a national nicotine reduction policy.

2.4. Summary and Rationale

This study will examine the impact of very low nicotine content (VLNC) cigarettes in a complex tobacco and nicotine product marketplace. We will compare the number of cigarettes smoked and smoke-free days, use of alternative nicotine-containing products, biomarkers of toxicant exposure and subjective responses to products in an experimental marketplace that contains VLNC cigarettes versus normal nicotine content cigarettes. The results of this study would contribute to policy decisions on whether or not nicotine levels should be regulated in cigarettes.

3. Study Endpoints / Outcomes

3.1. Primary Endpoints

- Cigarettes per day (CPD): the mean cigarettes (study and non-study cigarettes) smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit.
- Smoke-free days: the number of smoke-free days during Phase 3 based on IVR-Revised.

3.2. Secondary Endpoints

- Biomarker: percent change in biomarker, CEMA at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Study Cigarettes per day (CPD): the mean study cigarettes smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit.
- Seven-day abstinence and CO ≤ 6ppm for all 7 days before the week 12 visit.

3.3. Exploratory Endpoint:

- Non-combusted tobacco/nicotine products use: the number of days using any non-combusted tobacco/nicotine products in Phase 3.
- Characteristics' of products chosen (type, flavor, nicotine strength)
- Biomarkers: percent change in biomarkers (Total Nicotine Equivalents, total NNAL, mercapturic acids other than CEMA) at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Product satisfaction (Product Evaluation Scale if they used the product since the last visit; Cigarette Evaluation Scale if they smoked cigarettes since the last visit)
- Perceived health risk (Phase 1, Phase 2 and end of week 12 of Phase 3: all products for all subjects)
- Measures of discomfort/dysfunction: MNWS-R, QSU, CESD (NNC vs VLNC cigarettes irrespective of use of products)
- Dependence (FTND, WISDM, PATH items; monthly for study cigarettes)

3.4. Safety Endpoints:

- Potential adverse consequences: Change in mental (CES-D) or physical health (heart rate, blood pressure, weight)
- Increased TNE (corrected by creatinine), calculated as change from Phase 1 baseline.
- Adverse events and Serious Adverse events

4. Intervention

4.1. Study Description

This randomized, open label, controlled, multi-site study will simulate a "real world" tobacco environment by providing participants access to an experimental marketplace where they will be given vouchers for a specified number of points that can be exchanged for study cigarettes (varying in nicotine content described below) and non-combusted tobacco/nicotine products (smokeless tobacco, snus, nicotine pouches, electronic cigarette, medicinal nicotine replacement) or cash. More specifically, subjects (N=approximately 200-225 in each group) will be randomly assigned to: 1) very low nicotine content cigarettes (VLNC; 0.4 mg/g) along with non-combusted tobacco/nicotine products or 2) normal nicotine content cigarettes (NNC; 15.8 mg/g) along with non-combusted tobacco/nicotine products.

4.2. Investigational Products

4.2.1. Investigational Cigarettes and Marketplace Tobacco Products:

Study Cigarettes: Spectrum cigarettes will be ordered through NIDA (NOT-DA-14-004) and dispensed by Research Triangle Institute (RTI) to Wake Forest University, who holds a

distributer license and will distribute study cigarettes to sites. The product characteristics including filter ventilation and filter and paper type are the same across these two products.

The following nicotine content and yield will be obtained from NIDA:

Type	Nicotine Yield mg/cig	Tar Yield mg/cig
NNC600	0.8 ± 0.15*	10 ± 1.5
NNC601 Menthol	0.8 ± 0.15	10 ± 1.5
VLNC102	0.03 ± 0.01^	9 ± 1.5
VLNC103 Menthol	0.03 ± 0.01	9 ± 1.5

NNC= conventional nicotine; VLNC=very low nicotine content

*This NNC dose was chosen because it is similar to commercial cigarettes and is associated with similar rates of smoking, nicotine levels and dependence as usual brand cigarettes. [14]

^This dose was chosen because prior studies have shown that the VLNC dose relative to NNC dose showed significant reductions in cigarettes smoked per day, nicotine exposure and cigarette dependence.[14,17]

The selection of non-combusted tobacco/nicotine alternative nicotine delivery systems (ANDS) will include:

- E-cigarettes or electronic nicotine delivery systems (ENDS) that are commercially available and of varying nicotine doses and types (e.g., cigarette-like/pod systems such as Vuse, Juul and self-contained tank system such as Joytech eGo AIO or Halo Triton or similar;
- Snus (e.g., Camel, Skoal);
- Nicotine pouches (e.g. Zyn);
- Conventional smokeless tobacco products (e.g., Copenhagen, Grizzly);
- Non-prescription medicinal nicotine (2mg and 4mg nicotine gum and lozenge; 7, 14 and 21 mg nicotine patch).

The most popular brands, types and flavors (no more than 4 flavors for each type of product) will be chosen for our marketplace. The selection of products will be based on current national marketing data as well as data collected from the six study site regions, but the final product selections will be consistent across sites. The ENDS market is dynamic and changes may occur during the course of the study and as a result, specific brands will not be specified. Although oral tobacco products and medicinal nicotine are not the most popular products chosen in our pilot study, the products are included to more closely simulate the products that are currently on the market and because we may find they are preferentially chosen at some sites or in a sub-sample of smokers. Products made available in the study marketplace will be reassessed on a yearly basis or as product availability and safety/quality issues arise).

Experimental marketplace. The overall goal of the experimental marketplace is to provide a context in which product preferences can be evaluated in a systematic way but with a high degree of ecological validity. Participants will exchange points for products in the marketplace. Points required to exchange for products will vary from site to site to reflect regional variation in pricing, however, points required for products within a site will be the same.

Product pricing. Retail market value of all products is determined by recording product prices at a range of retail outlets (e.g. pharmacy, grocery, gas station, corner shop, tobacco outlet, vape shop) at each site. The price of usual brand cigarettes and Spectrum study cigarettes, which are not available retail, will be equal to the site-specific average cost of cigarettes. We will annually assess retail prices and modify study product pricing to reflect significant changes (>10% difference). All products will be discounted to 66% of the regional retail market value in order to 1) discourage subjects from purchasing products outside the marketplace and 2) minimize hoarding vouchers for cash payment at the end of the study.

Purchasing power. Each subject will be given a point voucher at each marketplace visit that can be exchanged for products. Points provided at each visit are based on the subject's points per day multiplied by the number of days till their next scheduled visit. Subject's points per day are constant throughout the study and are calculated to allow the subject to cover their baseline cigarettes and other nicotine product use (e.g., vaping device, smokeless tobacco or nicotine replacement therapies) per day plus 1 point per day to allow for experimentation in the marketplace. Participants will receive a coupon that can be applied to discount e-cigarettes and/or medicinal nicotine products in the marketplace. This will allow for experimentation with the more expensive e-cigarette devices or a large box of medicinal nicotine. This coupon cannot be used on cigarettes or smokeless tobacco/snus/nicotine pouches. This coupon can only be used one time per participant. The participant cannot exchange the coupon for cash at the end of the study. Although the need for higher voucher point amounts to take into account the possibility of compensatory smoking may be a concern, to date, the majority of people do not increase in smoking resulting from availability of VLNC cigarettes. In order to discourage needless exchange of vouchers for products, any portion of the voucher not used at each visit can be banked and used at later visits or converted to cash (up to $200) at the end of the study. Unopened products will be reimbursed for the amount of points exchanged for the product.

Marketplace protocol. The marketplace will have the appearance of an on-line virtual retail store, with points posted on each of the product brands/types but do not have any point-of-sale advertisements. Subjects will be informed that they can exchange points for any amount or type of product up to the value of current and banked vouchers. The Marketplace website will allow for standardized information to be available when the participant clicks on the "information" option. This standardized information will include the general contents of the product (flavor) and the levels of nicotine content and warning labels (no more information than what is typically provided by the retailer). Additionally, the subject can click on the product and observe a video describing how to use the product. For medicinal nicotine products, subjects will also be referred to consult the information/instructions provided in the package. Examples of the products will be available for the subject to inspect, smell and see that packaging. Post-COVID, this procedure was not possible, but participants can ask the Research Assistant for additional description of the product if necessary. If the subject requests additional information, s/he will be directed to the product's website. If the subject asks directly about e-cigarette risks, the Research Assistant will reply "The Surgeon General has said that long term risks of e-cigs are unknown, but it is likely less harmful than cigarettes. However, this is likely to be the case if the e-cigarettes and e-fluids are bought at reputable sources and not modified" If the inquiry is

related to smokeless tobacco or snus, the Research Assistant will reply that smokeless tobacco is not harmless, but it is less harmful than cigarettes. If the inquiry is related to medicinal nicotine, the Research Assistant will reply that medicinal nicotine is approved by the FDA for the treatment of smoking cessation and has minimal risks compared to smoking.

Outside purchases. If subjects run out of products between visits and wish to purchase more with banked vouchers, they can arrange for between-visit purchases with study staff during office hours. At these additional visits, safety measures may be collected (e.g. adverse events, vital signs, CO, and product accountability measures, as warranted). CO will be obtained if the cigarettes smoked per day increases by 50% or more. While subjects will be discouraged from making out-of-marketplace purchases of specific products offered in the marketplace, no penalties will be levied for such purchases in order to encourage truthful reports of noncompliance.

4.2.2. Investigational Tobacco Product Handling

Study cigarettes will be kept in locked refrigerators on site in a secured area with limited access. Refrigerator temperature and humidity will be documented on workdays to verify stability of environment. Only study staff will have access to open the refrigerator locks. Cartons will be pulled as needed for dispensing to subjects. Cartons will be labeled with the nicotine dose identified since this is not a blinded study. Cartons will be tracked by carton number and this number will be documented in the central Dispensing Log and in the subject case report form. Other tobacco and nicotine products will be kept in a secured location where only study staff will have access. Dispensing Logs will also be kept for these products.

4.3. Investigational Tobacco Product

This investigational cigarette to be used in this protocol is under FDA Submission Tracking Numbers: IU 0000011 (NRC600), 0000012 (NRC601), 0000126 (NRC 102), 0000127 (NRC 103).

This IU is held by the Project Leader, Dorothy Hatsukami, Ph.D.

5. Study Plan and Procedures

5.1. Study Design

This is a randomized, open label, controlled, multi-site study simulating a “real world” tobacco environment. Subjects are given access to an experimental marketplace where they will be given vouchers that can be exchanged for study cigarettes and non-combusted tobacco/ nicotine products or cash. Subjects (N=~200-225 in each group) will be randomly assigned to either:

1) Very low nicotine content cigarettes (VLNC; 0.4 mg/g, 0.03 nicotine yield) along with non-combusted tobacco/nicotine products

2) Normal nicotine content cigarettes (NNC; 15.8 mg/g; 0.8 nicotine yield) along with non-combusted tobacco/nicotine products.

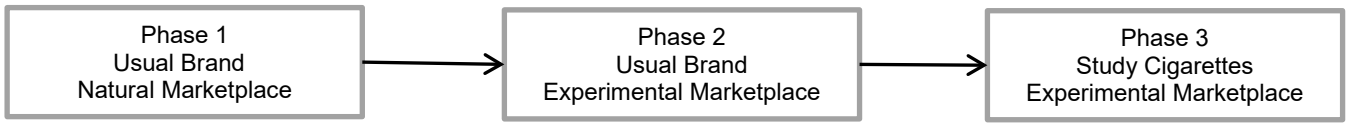
Subjects will be informed about the nicotine content in cigarettes they are assigned. Participants will have the option to select menthol or non-menthol cigarettes.

Smokers will undergo an orientation visit for further screening and then enter a three phase experimental trial:

1) Phase 1 – Baseline: a baseline assessment period during usual brand cigarette smoking.

2) Phase 2 – Marketplace Adaptation: a marketplace with access to the preferred usual brand cigarettes and selected non-combusted tobacco products to allow the subject to adjust to the marketplace prior to randomization;

3) Phase 3 – Intervention: randomization to a marketplace with access to either VLNC or NNC cigarettes and non-combusted products.



5.2. Clinic Visit Procedures

(Adaptation for COVID-19 safety precautions: In general, in-person visits converted to telehealth visits, including CO and vitals measurements using devices sent to participants. Product/biological sample drop-off and pick-up are conducted curbside. Some measures were discontinued including the breath alcohol test, toxicology screens for drug use, saliva samples were collected instead of spot urines for compliance, and first morning void was used for pregnancy screen.)

ELIGIBILITY SCREENING

5.2.1. Orientation and In-Person/Telehealth Screening (94)

(These 2 visits can be combined)

Orientation: The purpose of the orientation is to inform the subjects about the study protocol. During this meeting, subjects will be shown a standardized presentation on the study rationale, description of the cigarettes and alternative nicotine products, study protocol and outcome measures. A copy of the consent form will be provided for review. Interested subjects will be asked to contact the clinic to schedule an In-Person/Telehealth Screening visit, if the Orientation and In-Person/Telehealth Screening are done in two separate visits.

In-Person/Telehealth Screening: Subjects will first present a valid photo ID prior to obtaining informed consent to confirm age and identity. After informed consent is obtained (See Section

21.1 for consenting process), the following measures will be collected and documented in the subject's case report form:

A. Physiological Measures at Screening

1) Breath alcohol concentration (BrAC) measured by an expired breath sample. Participants with levels over 0.010 g/210L may reschedule the interview once but will need to be re-consented to ensure the subject is not intoxicated while giving informed consent. (BrAC was discontinued due to the COVID-19 pandemic to allow for telehealth visits.)
2) Expired breath carbon monoxide (CO) level to assess recent smoking. Carbon monoxide level must be ≥10 ppm to confirm regular daily smoking.
3) Vital signs (blood pressure and heart rate).
4) Height and weight. For Telehealth visits, weight was by self-report.
5) Spot urine sample:
    a. Verification of smoking status: If CO is <10, a cotinine (nicotine) strip can be used to assess urinary cotinine quantity to verify participant is a smoker. (First morning void used due to COVID-19 pandemic to allow for telehealth visits.)
    b. 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. A failed drug screen (with the exception of marijuana or valid prescription for other drugs) will result in discontinuing the visit without compensation. The subject may reschedule the screening visit, but will need to be re-consented to ensure the subject is not under the influence while giving informed consent. They will be excluded if positive again at the second visit. Marijuana use, as determined by medical professional review, is acceptable, due to its high prevalence; however, 48 hours abstinence from smoked marijuana will be requested prior to biomarker collection visits. If a subject reports smoked marijuana within 48 hours of the visit it will be documented. (The toxicology screen for eligibility was discontinued due to the COVID-19 pandemic to allow for telehealth visits.)

B. Questionnaires and Assessments at Screening **(See section 5.7.3 – 5.7.5 for further descriptions of questionnaires)**

B.0 Pen and paper forms (These forms were later completed electronically during the Telehealth visits)

Pen and paper questionnaires will be completed by the subject and some of this data will be entered into REDCap following the visit (identified in the Schedule of Procedures Table on page 65).

1) Identification Form: includes name, address, email, phone number, age and date of birth. This information will be stored separately from any other study data and in a password protected database. This information will not be shared with any study investigators external to the site.

2) Brief Medical History Questionnaire to assess current diagnoses, symptoms and past health problems including psychiatric and substance abuse and medication use.
   - Date of last menstrual period and length of cycle are included to assess pregnancy status. (A pregnancy test [hCG detection] will be performed for female participants of childbearing potential prior to accessing the Marketplace at week 92 and prior to randomization at week 00 and monthly thereafter).
3) Patient Health Questionnaire (PHQ - PrimeMD).
   - Beck Depression Inventory 2nd Edition (BDI-II) administered for clinician review if the PrimeMD is positive for any diagnoses or endorsement of suicidal ideation.
   - GAD-7 will be administered if the PrimeMD is positive for anxiety/panic disorders.

B.1 Web-based data collection completed by the subject:

1) Demographics.
2) Contemplation Ladder.
3) Michigan Alcohol Screening Test Short form (SMAST).
4) Drug Abuse Screening Test (DAST).
5) NIAAA Alcohol Use Questionnaire (12 month version).
6) Centers for Epidemiological Studies–Depression 20-item scale (CES-D).
   - BDI-II will be administered on paper if the CES-D score is 16 or greater for clinician review.

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Brief Medical History Follow-up Questionnaire (not entered in REDCap) will be completed by study staff to further assess current medical diagnoses, symptoms or past health problems.
2) Tobacco Use History and Nicotine Exposure Questionnaire.
3) The Mini International Neuropsychiatric Interview (MINI).
   - Any response other than “not at all” on the suicidal ideation question of the Prime MD (Question 1i) or a positive response to items 2-5 on the (MINI) suicide subscale will not be eligible to participate in the study and will be assessed by clinician, and provided with information and resources and dismissed from the study.
   - If a subject is in immediate danger, site’s procedures for suicidal subjects will be followed.
4) Recreational Drug Use Questionnaire (12 month version).
5) End of Visit Form.

After confirmation of eligibility criteria, subjects will be scheduled for their first baseline visit and medical review will be completed by the licensed medical professional (LMP) prior to the next visit. If upon medical review, it is determined the subject is not eligible, they will be contacted by phone and informed.

C. Daily Call Recording System - Interactive Voice Response System (IVR)

At the Screening visit, eligible subjects will be trained to use the IVR Daily Call Recording System, which will contact participants each day throughout the study and ask about their

smoking and other tobacco/nicotine product use. The questionnaire will ask for previous day's use of cigarettes (study and non-study cigarettes), number of vaping puffs, number or alternative smokeless tobacco and nicotine products used.

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

Any missing data due to incomplete calls or entry errors (e.g., subject entered 88 study cigarettes rather than 8 study cigarettes) will be reviewed at the visit via the Timeline Followback Method and compiled in the IVR Review Form. This data will be integrated into the IVR-Revised data, however initial data will be retained in the original IVR database.

Visit Scheduling Requirements

The scheduling window for baseline visits is 7 days + 14 days. Subjects will be required to schedule the Baseline 1 (93) visit within 21 days of their In-Person/Telehealth Screening (94) visit. If the first baseline visit is over 30 days after screening due to scheduling or eligibilitly criteria, the subject must be re-consented and re-screened, but will maintain the original assigned Subject Identifier.

PHASE 1: BASELINE: USUAL BRAND IN NATURAL MARKETPLACE

5.2.2. Phase 1: Baseline Procedures for Baseline Visits 1 (93) and 2 (92)

During Phase 1 of baseline (screening up to access to tobacco marketplace), eligible subjects will continue to smoke their preferred brand as usual. At the baseline clinic visits, subjects will complete several measures and provide biomarker samples as described below.

A. Physiological Measures at Baseline (93 and 92):

1) BrAC, which must be below 0.03 g/210L. If over the 0.03, the visit will be delayed until the level drops within range or subject can be rescheduled. If above 0.05, the visit will be rescheduled. Multiple baseline elevated BrAC results may result in discontinuation of the subject at the PI discretion. (BrAC was discontinued in conversion to telehealth visits.)
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) First morning void urine collected.
6) Saliva samples:
   a) Saliva sample obtained with Salivette (with citric acid preparation) to be used for nicotine metabolite ratio (NMR) Visit 1 (93) only.
7) Spot urine for pregnancy tests [hCG detection] will be performed for female participants of childbearing potential (not surgically sterilized or 2 years post-menopausal) at Week

92 prior to offering Marketplace. (Pregnancy tests are performed on first morning void after conversion to telehealth visits.)

B. Questionnaires and Assessments at Baseline (93 and 92):

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges- Brief - Usual Brand Cigarette (QSU).
3) Cigarette Evaluation Scale – Baseline (CES – Usual Brand).
4) Respiratory and Global Health Questionnaire.
5) If monitored for psychiatric illness: Beck Depression Inventory 2nd Edition (BD-II) (paper only).
6) Cigarette Dependence Measures (Usual Brand - Visit 93 only).
7) Environmental Tobacco Smoke Exposure Questionnaire (Visit 93 only).
8) Social and Environmental Influences on Tobacco Use (Visit 93 only).
9) Dietary Intake (e.g., charred and smoked meats and fish) (Visit 93 only).
10) NIAAA Alcohol Use Questionnaire - 1 month version (Visit 93 only).
11) Expected Utility (ANDS) (Visit 93 only).
12) Perceived Health Risk of no tobacco use, usual brand cigarettes, very low nicotine cigarettes, smokeless tobacco/snus/nicotine pouches, medicinal nicotine, ENDS (Visit 92 only).
13) Product Expectancy Questionnaire (Visit 92 only).
14) Cigarette (Usual Brand)/Product Purchase Task (Week 92 only).

B.2. Administered as an interview by the Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) IVR Review of Daily Call and follow-up on missing and unusual reports.
5) Timeline Followback Questionnaire (14 days for Visit 93 or since last visit for Visit 92).
6) Recreational Drug Use Questionnaire (1 month version at Visit 93).
7) Study Product (see C.1-3: Visit 92 only) and Procedure Compliance Review.
8) End of Visit Evaluation.

C. Introduction to Experimental Marketplace with Usual Brand Cigarettes:

Upon completion of V92 (end of Baseline), subjects will have access to the web-based experimental marketplace that includes their usual brand cigarettes and non-combusted tobacco and nicotine products. This phase is instituted to allow the participant to adjust to the experimental marketplace while smoking usual brand cigarettes prior to randomization

to study cigarettes. In addition, exploratory comparisons will be made between Phases 1 and 2 to assess purchasing and product use patterns when participants are purchasing their own cigarettes in the natural environment versus when purchasing products in the experimental market.

C.1 Present marketplace web page

Subjects will be presented with the Marketplace web page containing their usual brand cigarettes and the other study tobacco and nicotine products (no study cigarettes). These products include: smokeless tobacco, snus, nicotine pouches, electronic nicotine delivery systems (ENDS) and medicinal nicotine. The point exchange system will be reviewed and the subjects are shown how to access product information on the webpage. They will then choose their preferred products and are instructed to only use the products they put in their "cart" in exchange for their points.

C.2 Dispense Study Product

After subject has selected their desired products (usual brand cigarettes and alternative nicotine products) from the Marketplace website, those products will be provided to the subject with instruction on how to track the use of their products for the Daily Call Recording System.

C.3 Product Accountability (Dispensing Log)

Any products obtained by the subject will be documented in the Product Accountability Log in the subject's case report form. Subjects will be asked to return used and unused product packaging and empty vaping device cartridges/e-liquid containers. Number of products dispensed, used and unused items will be documented at each visit. If smokeless tobacco products are chosen, the tins will be weighed before dispensing and after return. A similar procedure will be followed for vials of e-fluids that are dispensed. Review compliance counseling with the study product use and procedures.

(Refer to Section 4.2.1: Marketplace protocol. *for additional information)*

PHASE 2: USUAL BRAND IN EXPERMENTAL MARKETPLACE (MP91 AND MP00)

Scheduling window at baseline is 7 days + 4 days.

During the 2 weeks of Phase 2 (MP 91 and MP 00), eligible subjects will have access to the marketplace products that contains their usual brand cigarettes and other tobacco products. At this visit, subjects will complete several measures and provide biomarker samples as described below.

5.2.3. Phase 2: Marketplace Adaptation Visit MP (91) Procedures

A. Physiological Measures at MP 91:

1) BrAC (discontinued at telehealth visits).
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.

B. Questionnaires and Assessments at MP 91:

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges-Brief -Usual Cigarette (QSU).
3) Cigarette & Product Evaluation Scale – (Baseline Usual Brand CES/PES).
4) Respiratory and Global Health Questionnaire.
5) If monitored for psychiatric illness: Beck Depression Inventory 2nd Edition (BD-II) (paper only).

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Review of Daily Call and follow-up on missing and unusual reports.
5) Timeline Followback Questionnaire (since last visit).
6) Product Accountability: Complete Product Dispensing Log.
7) Study Product and Procedure Compliance Review.
8) End of Visit Questionnaire.

5.2.4. Phase 2: Marketplace Adaptation Visit (00) Procedures

***A. Physiological Measures at MP 00*:**

1) BrAC (discontinued at telehealth visits).
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) First morning void urine.
6) Spot urine for pregnancy tests [hCG detection] will be performed for female participants of childbearing potential (not surgically sterilized or 2 years post-menopausal) at Week 00. (Pregnancy tests are performed on first morning void after conversion to telehealth visits.)

***B. Questionnaires and Assessments at MP 00**:*

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges-Brief -Usual Cigarette (QSU).
3) Cigarette & Product Evaluation Scale – (Baseline Usual Brand CES/PES).
4) Respiratory and Global Health Questionnaire.
5) CES-D:

   Beck Depression Inventory 2nd Edition (BD-II) will be administered on paper if the CES-D score is 16 or greater of if monitored for psychiatric illness.
6) Cigarette Dependence Measures (Usual brand)
7) Environmental Tobacco Smoke Exposure Questionnaire
8) Dietary Intake.
9) NIAAA Alcohol Use Questionnaire (1 month).
10) Perceived Health Risk of no tobacco use, usual brand cigarettes, very low nicotine cigarettes, smokeless tobacco, medicinal nicotine and ENDS.
11) Contemplation Ladder.
12) Cigarette (Usual Brand and All Products) Purchase Task.

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Review of Daily Call and follow-up of missing and unusual reports.
5) Timeline Followback Questionnaire.
6) Recreational Drug Use Questionnaire (1 month version).
7) Product Accountability: Complete Product Dispensing Log.
8) Study Product and Procedures Compliance Review.
9) End of Visit Questionnaire.

Randomization and Marketplace with study cigarettes

At the end of Visit 00, subjects will be randomized to one of the two experimental conditions (VLNC vs. NNC cigarettes) for 12 weeks. Twelve weeks was chosen because experimentation with or use of other tobacco/nicotine products is most likely to occur during the first weeks of intervention and stabilization is likely to occur thereafter. However, the time course of stabilization of product use is not clearly known, a 12 week duration of intervention was selected to allow sufficient time to observe any smoking cessation behaviors.

Subjects will be informed of the general nicotine content of the Spectrum cigarettes. Subjects assigned to the VLNC Spectrum cigarette condition will be informed they contain minimal amounts of nicotine to reduce their addictiveness and those assigned to the NNC Spectrum cigarettes will be informed that they contain levels of nicotine that are similar to the

conventional cigarettes that are currently on the market. Research cigarettes are provided under non-blind conditions because if a reduced-nicotine policy were to be implemented, the FDA would by necessity provide information to the public on the reduced nicotine content of cigarettes.

PHASE 3: INTERVENTION: STUDY CIGARETTES IN EXPERIMENTAL MARKETPLACE

Scheduling window at baseline is 7 days -3/+ 4 days and 14 days -6/+ 7 days for bi-weekly visits.

During the Phase 3 visits (Weeks 1-12), subjects will continue to acquire the study cigarettes and alternative nicotine products via the Marketplace website (no access to usual brand cigarettes). At the clinic visits, subjects will complete several measures and provide biomarker samples as described below.

5.2.5. Procedures for Phase 3 - Weeks 1, 2, and 3 (Week 3 was discontinued following telehealth procedure changes)

At these visits, the procedures will only involve access to products and checking records of product use and safety checks.

A. Physiological Measures at Weeks 1, 2 and 3:

1) BrAC (discontinued at telehealth visits).
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) Spot urine sample for compliance testing. (Saliva samples for compliance testing are collected at telehealth visits.)

B. Questionnaires and Assessments at Weeks 1, 2, and 3:

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges-Brief - Study Cigarette (QSU).
3) Cigarette & Product Evaluation Scale – (Study Cigarettes CES/PES).
4) Respiratory and Global Health Questionnaire.
5) If monitored for psychiatric illness: Beck Depression Inventory 2nd Edition (BD-II) (paper only).

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.

3) Concomitant Medications.
4) Review of Daily Call and follow-up on missing and unusual reports.
5) Timeline Followback Questionnaire (since last visit).
6) Study Product Accountability: Complete product dispensing log.
7) Study Product and Procedures Compliance Review.
8) End of Visit Questionnaire.

5.2.6. Procedures for Phase 3 - Weeks 4, 8 and 12

A. Physiological Measures at Weeks 4, 8 and 12:

1) BrAC (discontinued at telehealth visits).
2) CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) First morning void urine sample.
6) Spot urine for pregnancy tests [hCG detection] will be performed for female participants of childbearing potential. (Pregnancy tests are performed on first morning void after conversion to telehealth visits.)
7) Spot urine sample for compliance testing. (Saliva samples for compliance testing are collected at telehealth visits.)
8) Drug screen (Week 12 only). (Discontinued at telehealth visits.)

B. Questionnaires and Assessments at Weeks 4, 8 and 12:

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges-Brief-Study Cigarette (QSU).
3) Cigarette & Product Evaluation Scale – (Study Cigarettes CES/PES).
4) Respiratory and Global Health Questionnaire.
5) CES-D:

   Beck Depression Inventory 2nd Edition (BD-II) will be administered on paper if the CES-D score is 16 or greater or if monitored for psychiatric illness.
6) Cigarette Dependence Measures (Study Cigarettes).
7) Environmental Tobacco Smoke Exposure Questionnaire.
8) Dietary Intake.
9) NIAAA Alcohol Use Questionnaire (1 month).
10) Social and Environmental Influences on Tobacco Use (Week 12 only).

11) Perceived Health Risk of no tobacco use, usual brand cigarettes, study cigarettes, smokeless tobacco, medicinal nicotine and ENDS (Week 12 only).

12) Cigarette/Product Purchase Task (Week 12 only).

13) Vaping/Product Dependence Scale (week 12 only)

B.2 Administered as an interview by the Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Review of Daily Call and follow-up of missing and unusual reports.
5) Timeline Followback Questionnaire (since last visit).
6) Recreational Drug Use Questionnaire (1 month version).
7) Study Product Accountability: Complete Product Dispensing Log.
8) Study Product and Procedures Compliance Review.
9) End of Visit Questionnaire.

5.2.7. Procedures for Phase 3 - Weeks 6 and 10

At these visits, the procedures will only involve access to products and checking records of product use and safety checks.

A. Physiological Measures at Weeks 6 and 10:

1) BrAC (discontinued at telehealth visits).
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) Spot urine sample for compliance testing. (Saliva samples for compliance testing are collected at telehealth visits.)

B. Questionnaires and Assessments at Weeks 6 and 10:

B.1 Web-based data collection program completed by the subject:

1) Minnesota Nicotine Withdrawal Scale (MNWS).
2) Questionnaire of Smoking Urges-Brief-Study Cigarette (QSU).
3) Cigarette & Product Evaluation Scale – (Study Cigarettes CES/PES).
4) Respiratory and Global Health Questionnaire.
5) If monitored for psychiatric illness: Beck Depression Inventory 2nd Edition (BD-II) (paper only).

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes and Menstrual Cycle Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Review of Daily Call and follow-up on missing and unusual reports.
5) Timeline Followback Questionnaire (since last visit).
6) Study Product Accountability: Complete product dispensing log.
7) Study Product and Procedures Compliance Review.
8) End of Visit Questionnaire.

5.3. Study Product and Procedures Compliance Review Sessions

At each study product visit, subjects will be counseled about use of only products obtained through the experimental Marketplace website. Subjects will be asked about any concerns or obstacles associated with use of the study products. The importance of honest reporting will be stressed. Subjects will be told that it is crucial for them to report any use of products obtained from outside the experimental marketplace, even though it is discouraged. If difficulties using only study products are encountered, they will be asked why they are experiencing difficulties (e.g., taste, withdrawal symptoms) and problem-solve how to deal with these difficulties in order to meet the protocol requirements. Additionally, compliance counseling will include daily diary call completion, visit attendance and accurate product accountability. These review sessions should be about 5-10 minutes in length.

5.4. Quit Attempts During the Study Protocol:

At each session, we will ask if the subject is currently abstaining from smoking and/or has the intention of quitting or planning to quit smoking or tobacco use prior to the next scheduled visit. If the subject chooses to quit during the study, they will be encouraged to do so and will continue to receive vouchers.

If Currently Abstinent from Smoking:

- Encourage continued abstinence from smoking.
- Provide the cessation manual and local smoking cessation resources.
- Remind the subject that medicinal nicotine is available in the Marketplace and may aid in their cessation efforts.
- Schedule normal weekly or bi-weekly visits.

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

- Ask if a target quit date has been chosen and document the target quit date.
- Provide the participant with the cessation manual and local smoking cessation resources.
- Remind the subject that medicinal nicotine is available in the Marketplace and may aid in their cessation efforts.

- Recommend that on the target quit date he/she puts any tobacco products “away” so as to avoid unwanted cues to smoke

At the end of the 12 week experimental period, all subjects will be strongly encouraged to stop smoking and if possible, using all tobacco products, and set a quit date. A treatment manual for cessation will be provided and subjects will be encouraged to call the state telephone quit line. If the subject has a quit date planned or intends to set a quit date they may obtain medicinal nicotine from Marketplace with any remaining points. No additional points are given at this visit.

5.5. Tobacco/nicotine use measures: Consistency and product compliance

The importance of honest reporting will be stressed. Consistency across data collection will be examinined by comparing: a) daily call records; b) timeline follow-back; and c) product accountability logs where the amount of products dispensed are recorded and any unused products and packages/ cartridges from used products returned to the clinic are recorded. For smokeless tobacco, we will weigh tins prior to distribution and after use. A similar procedure will be followed for vials of e-fluids that are dispensed. Participants will be allowed to keep any of the unused products until the follow-up visit. Participants will be provided feedback regarding the consistencies across data collected and encouraged to be more accurate if the data is inconsistent. In the event that subjects purchased a study product outside our experimental market or obtained product by other means (e.g., provided by a friend), they will be asked to report the type and amount of product to us without penalty. (Purchase of combustible products would be penalized; see compliance section.) Several biochemical methods will be used to confirm product self-report: 1) urinary anatabine < 0.01 nmol/ if a subject is assigned to VLNC cigarettes and reports use of only VLNC cigarettes, medicinal nicotine and/or ENDS; and 2) CO $\leq$ 6 ppm if a subject reports use of only non-combusted products. The level for urinary anatabine was determined by a study in which smokers were confined to a residential living situation and asked to only smoke VLNC study cigarettes.[27] Data will be analyzed with and without this biochemical verification. Unfortunately, biochemical analysis is not available to confirm use of VLNC cigarettes when other tobacco products are also used (i.e., smokeless tobacco) nor to distinguish usual-brand cigarettes from NNC study cigarettes. But non-compliance is less likely in the NNC condition given that rates of usual-brand and NNC smoking were nearly identical in our prior study.[14] Reports of use of non-study cigarettes will be considered as an outcome variable in this study and as a potential indicator of a need to supplement nicotine via another source.

5.6. Follow-up

5.6.1. Procedures for Week 16

Follow-up will occur 4 weeks (-7 and + 30 days) after the end of the experimental period. Tobacco use status (amount and type of tobacco product use) will be determined and first void urine samples assessed for various biomarkers of exposure, partially as a safety assessment. Quitting smoking or staying quit will continue to be strongly encouraged.

A. Physiological Measures at Week 16:

1) BrAC (discontinued at telehealth visits).
2) Expired breath CO level.
3) Vitals signs (blood pressure, heart rate).
4) Weight.
5) First morning void urine sample.
6) Collect vaping device and smokeless tobacco products, if obtained from the experimental marketplace during study.

B. Questionnaires and Assessments at Week 16:

B.1 Web-based data collection program completed by the subject:

1) Cigarette Evaluation Scale - Usual Brand.
2) Respiratory and Global Health Questionnaires.
3) CES-D;

   Beck Depression Inventory 2nd Edition (BD-II) will be administered on paper if the CES-D score is 16 or greater or if monitored for psychiatric illness.
4) Cigarette Dependence Measures (Usual Brand).
5) End of Study Questionnaire.

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Timeline Followback Questionnaire (since last visit) Follow-up version.
5) End of Visit Questionnaire.

5.6.2. Procedures for Post-Follow-up

If urinary biomarker levels from the Phase 3 Week 16 visit are substantially elevated over baseline levels and it is determined by the PI that this may be a safety issue, subjects will be sent a letter to alert them of their nicotine exposure, advise them to try to reduce their tobacco/nicotine use, and they will be strongly encouraged to quit smoking and provided tobacco cessation resources. If subjects have any questions, they are free to contact us.

5.6.3. Study Completion

Any Adverse Event or Concomitant Medications that remain open from the last study session will be assessed and closed or indicated as ongoing at end of study. Once a participant has completed all study procedures and all open events have been closed or indicated as ongoing

at end of trial, the Site Leader will review the participant's binder and sign off indicating study completion for that participant.

5.7. Data Collection Measures

5.7.1. Individually Identifiable Health Information

No information will be collected from medical records. Identifiable information will be retained at each site and will only be available to the local study and regulatory staff. The only exception is subject telephone numbers provided to a HIPAA compliant vendor for our Interactive Voice Response System.

5.7.2. Data Collection Platforms

Data will be collected and entered into multiple platforms including the REDCap, which will be used by RAs to enter data and by subjects to directly enter their responses to questionnaires, and the Daily Call Interactive Voice Response System (IVR) on a HIPAA secured website. In the event that internet access is not functioning, the assessments will be printed out and administered on paper. The source documents will be kept in the participant's case report form binder and the interviewer will enter the data into the software when it resumes functioning properly. Biosamples will be entered into a biomarker tracking database as they are collected.

The following questionnaires and other measures will be collected throughout the study. Some forms are administered only at baseline to assess history and eligibility criteria and others are repeated measures:

5.7.3. Screening Questionnaires

1) *Tobacco Use History and Nicotine Exposure*, derived from the Population Assessment of Tobacco and Health (PATH) allowing comparisons with a nationally representative sample of smokers which measures variables such as smoking rate (current and maximum), cigarette brand, age of initiation of smoking, number of quit attempts, duration of quit attempts and duration of smoking and history of use of other tobacco and nicotine products.
2) *Demographics* asks about age and gender, race, ethnicity, current occupation and usual occupation, and income.
3) *Brief Medical History Questionnaire to* assess medical history and current health diagnoses, symptoms and past health problems including psychiatric and substance abuse and medication use.
    - Medical History Follow-up Questionnaire will be completed by study staff to further assess current diagnoses, symptoms or past health problems.
4) *Concomitant Medication*s lists medications (prescription, over-the-counter and supplements) and doses that are taken 30 days prior and during the study.
5) *PrimeMD - Patient Health Questionnaire (PHQ),* a brief multi-choice questionnaire developed as a screening and diagnostic tool for mental health disorders of depression and anxiety (somatoform, alcohol and eating disorder questions are not used in this study) and developed for use by primary care physicians.[28]

6) *Centers for Epidemiological Studies-Depression 20-item scale* (CES-D) which measures current symptoms of depression.[29]
7) Mini International Neuropsychiatric Interview (MINI) suicide subscale (43) to evaluate suicide risk.[30]
8) *Beck Depression Inventory 2nd Edition (BD-II)* is a severity measure assessing symptoms of depression.[31]
9) *Michigan Alcohol Screening Test Short form (SMAST)* is a widely used measure for assessing lifetime alcohol abuse.[32]
10) *Drug Abuse Screening Test (DAST)* is a brief instrument for population screening assessing lifetime drug abuse.[33]

5.7.4. Biomarker Modifier Questionnaires

Measures of factors that may moderate biomarkers of exposure and effect:

1) *Environmental Tobacco Smoke Exposure Questionnaire* consists of 1 question related to tobacco smoke exposure at home, work and socially.[34]
2) *Dietary Intake* assesses intake of specific foods high in polycyclic aromatic hydrocarbons (e.g., charred and smoked meats and fish).

5.7.5. Subjective Outcome Measures

1) *Interactive Voice Response – Daily Call Recording System* to record amount of product use, cigarettes, and other nicotine containing products on a daily basis (estimated ENDS puffs per day will be recorded),.
2) *Timeline followback* for tobacco[35] will be used as another measure of non-cigarette tobacco and nicotine intake), alcohol and marijuana use assessed at each clinic visit.
3) *IVR Review Form* will assess study and non-study use of cigarette and other products missing from the IVR calls, correct errors in IVR entries or clarify any discrepancies or unusual IVR reports.
4) *Cigarette/Vaping Dependence Measures* includes: Fagerstrom Test for Nicotine Dependence (FTND),[36] Penn State Cigarette Dependence Scale,[37] Brief Wisconsin Inventory of Smoking Motives (WISDM) primary dependence motives subscales[38] and 3 items from PATH.
5) *Severson Smokeless Tobacco (SLT) Dependence Questionnaire* is an 8 item measure to assess tobacco dependence.[39]
6) *Minnesota Nicotine Withdrawal Scale (MNWS)* is an 8 item assessing symptoms of tobacco withdrawal.[40]
7) *Questionnaire of Smoking Urges-Brief* 10 item measures the urge to smoke.[41]
8) *Adverse Events* to assess the nature, severity, duration, action taken, and outcome of adverse events related to tobacco/nicotine product use.
9) *Health Changes Questionnaire* queries for any health care visits or circumstances that would indicate any change since last visits.
10) *Respiratory and Global Health Questionnaire* rates overall health (1-10 scale) and respiratory symptoms such as cough, phlegm production, shortness of breath on a scale

ranging from 0 = none up to 10 = severe with a total respiratory score determined by adding the scores of each of these items.

11) *NIAAA Alcohol Use Questionnaire (1 and 12 month)* assesses frequency and quantity of alcohol use.[42]
12) *Recreational Drug Use History (1 and 12 month)* assesses amount, frequency in past year, and past month and date of last use of illicit drugs.
13) *Perceived Health Risks* assesses perceived health risks of no tobacco use, usual brand of cigarettes and study products that are provided.[43]
14) *Modified Cigarette Evaluation Scale*[44-46] *and/or Product Evaluation Scale*[47] assesses different dimensions of responses to cigarettes or other tobacco/nicotine products, (e.g., psychological reward, satisfaction, aversiveness, and additional oral and respiratory sensation items).
15) *Cigarette/Product Purchase Task* assesses demand curves of products that are chosen.[48,49] This task will be used to generate demand curves by asking the subject to report the number of units of a tobacco product that they would consume in a day if the units cost various amounts of money.
16) *Product Expectancy Questionnaire* to assess attitudes and beliefs about the products provided.
17) *Expected Utility (Alternative Nicotine Products) Questionnaire* assesses the situations or expected uses of the study products.
18) *Contemplation Ladder*[50] assesses intention to quit smoking including next 30 days and 6 months and overall readiness to quit.[51]
19) *Social and Environmental Influences on Tobacco Use* assesses tobacco/nicotine situational use and subject's opinion on potential tobacco control policies.
20) *End of Study Questionnaire,* administered at follow-up to assess subject responses to study products and study experience.

6. Collection and Sharing of Biomarker Specimens and Data

6.1. Storage and Access

Biomarker specimens described below will be collected and stored at study sites until shipment to the Masonic Cancer Center's Drs. Stephen Hecht, Irina Stepanov and Sharon Murphy laboratories for analysis or long term storage. Shipments will be requested at approximately quaterly intervals. Samples that are not used for the primary analysis of study biomarkers will be banked for future use. The banked samples will be stored until requested by a qualified investigator and used up or destroyed if it is determined they are no longer needed. The samples, including DNA or RNA, may be stored up to a maximum of 20 years from the study's end. A subject has the right to withdraw consent at any time by informing the Principal Investigator by following the instructions provided in the consent document. If this occurs, any remaining identifiable research sample(s) will be destroyed.

6.2. Data Storage

Local data will be stored on the Tobacco Research AHC server at the University of Minnesota. Upon completion of the trial, this data will be housed on the CENIC II portal which will be maintained by Clinical Informatics Shared Services at the University of MN. Shared data will be de-identified and includes demographics, tobacco/nicotine exposure history and current use, health and medications, subjective questionnaire data, biomarkers analyses, and visit number and intervention arm.

Biomarker samples that are banked after the completion of the primary analyses will be stored at the Masonic Cancer Center Hecht, Stepanov and Murphy laboratories located at the Cancer and Cardiovascular Research Building and Diehl Hall for future use.

6.3. Release/Sharing of samples

Access to the de-identified samples and data will be controlled by the Project 1 and the Biomarker Core investigators. Qualified researchers must submit an application request to access the samples and data to the Principal Investigator who will determine if the request is appropriate, in consultation with the other investigators.

6.4. Analysis of Biomarkers

The following panel of biomarkers has been selected to represent exposures to relevant toxicants.

Biomarkers of Carcinogen Exposure: Biomarkers include urinary NNAL (metabolite of NNK, a potent lung cancer carcinogen), mercapturic acid metabolites of acrolein, benzene, crotonaldehyde, and acrylonitrile. These biomarkers have shown good laboratory reproducibility, have clear differences in levels between smokers and nonsmokers and/or decrease upon tobacco cessation, and reflect differences in toxicants in the various tobacco/nicotine products.[52,53]

Oxidative damage: Urine samples will be banked for analysis of 8-iso-PGF2α and will be conducted if supplemental funding is received. If no funding is available the samples will be banked for sharing.

Total nicotine equivalents (TNEs): This is the molar sum of nicotine and its metabolites. TNE accounts for 73-96% of the nicotine dose and is a useful measure of daily nicotine exposure.

Nicotine metabolite ratio (NMR): NMR will be assessed from saliva as an indicator of CYP2A6 enzymatic activity and is the ratio between two nicotine metabolites, free cotinine and *trans*-3'-hydroxycontinine (3-HC). Because CYP2A6 is the primary enzyme associated with nicotine metabolism and also catalyzes the conversion of cotinine to 3-HC, the 3-HC:cotinine ratio is considered to reflect the enzymatic activity of CYP2A6 and therefore the extent of nicotine clearance rate (higher the ratio, the faster the clearance rate). This metabolite ratio has been observed to be moderately correlated to CYP2A6 genotype and highly correlated with the rate of nicotine metabolism.[54-59]

Genotyping sample: A saliva sample will be collected for potential genotyping for genes related to metabolism of tobacco constituents, behavior, mood, brain functioning and harmful effects of tobacco exposure. Subjects may opt out of this collection and still be allowed to participate in the study. If the subject decides to withdraw their samples during or after the study, the samples will be destroyed immediately.

Biobank: Other tobacco-related validated biomarkers for assessment in saliva or urines may be developed over time and these additional assays may be completed as appropriate using samples stored in the Biobank. Also, in the future, samples may be analyzed for genetic predisposition for tobacco toxicant metabolism, tobacco use behavior and tobacco-related harm. All samples remaining after the primary analyses will be biobanked.

7. Sharing Results with Subjects

No individual research or biomarker data will be shared with the subjects. Data that is collected at visits such as vital signs, carbon monoxide level, breath alcohol concentration test, drug screen results, will be evident to the subjects during the visits. If study medical staff believes that any of these measures are of clinical significance, such as observed worsening of psychiatric symptoms or subject reports of adverse events experienced during the trial that require further follow up, the subject will be referred to their primary care physician. Information will be released to the primary care physician or other health professional upon request of the subject.

No genetic testing information will be shared with subjects.

After data collection from all participants is complete and results are analyzed, participants may be mailed a letter telling them the general results of the study.

8. Study Duration

- A subject's direct study involvement is approximately 21 weeks. There may be some variation due to scheduling constraints. Baseline and intervention clinic visits are weekly for 9 weeks and bi-weekly for 8 weeks.
    - Screening - 1 week
    - Phase 1: Baseline - 2 weeks
    - Phase 2: Usual Brand Experimental Marketplace - 2 weeks
    - Phase 3: Study Cigarettes in Experimental Marketplace (Intervention) - 12 weeks
    - Follow-up: 1 visit 4 weeks post-intervention
- The duration of the study recruitment period will be approximately 47 months. (54 months with 7 month recruitment hiatus due to COVID-19.)
- The duration to complete all study procedures and initial data analysis is estimated to be 50 months. (55 months to complete the intervention and follow-up; an additional 5 months for primary data analysis and an additional 12 months for other analysis.)

9. Subject Selection

Study entry is open to adults who are healthy volunteers regardless of gender, race or ethnic background. While there will be every effort to seek out and include females and minority patients, the participant population is dependent upon the characteristics of the population at the sites where enrollment is occurring.

9.1. Inclusion Criteria

a) Male or female;
b) At least 21 years of age (legal age to buy tobacco; prior to 1/2021 at least 18 years of age at sites where legal age was 18);
c) Smoking at least 5 and no more than 40 cigarettes per day for the past month;
d) Biochemically confirmed regular smoking status by a carbon monoxide level of $\geq$10 ppm or if CO <10 ppm, a nicotine test strip that confirms smoking status.

9.2. Exclusion Criterion

a) Unstable health conditions (any significant serious, unstable medical condition including, but not limited to, cardiovascular disease, unstable COPD, seizure disorder (seizure within the last year), blood pressure, heart rate and cancer) as determined by the licensed medical professional at each site;
b) Unstable mental health (to be determined by medical history, CES-D, Prime-MD after review by the licensed medical professional at each site);
c) Suicidal Ideation in the past month. History of suicide attempts will be reviewed by the licensed medical professional to determine current risk;
d) Excessive drinking or problems with drinking or drugs (e.g., binge drinking more than nine days in the past 30 days) as assessed by PI or licensed medical professional;
e) Breath alcohol concentration (BrAC)> 0.01 as measured by breath sample at screening: Participants failing the BrAC screen will be allowed to re-screen once (discontinued due to COVID-19 pandemic when procedures were adapted for telehealth visits);
f) Positive toxicology screen for any of the following drugs: cocaine, opiates, methadone, benzodiazepines, barbiturates, amphetamines, methamphetamines, and PCP. Failing temperature strip for the sample. Marijuana use will be tested, but will only be an exclusionary criterion if the licensed medical professional decides that use will interfere with study results. Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines or methadone will not be excluded. Participants failing the toxicology screen will be allowed to re-screen once. (Toxicology screen was discontinued due to COVID-19 pandemic when procedures were adapted for telehealth visits);
g) Currently pregnant, breast feeding or intending to become pregnant for the duration of the study or unwilling to agree to use adequate protection to avoid pregnancy;
h) Currently taking any one of the following medications:
    1) Phenytoin [Brand Name: Dilantin] (if unstable)
    2) Carbamazepine [Brand Name: Tegretol, Carbatrol, Equetro, Epitol] (if unstable)
    3) Oxcarbazepine [Brand Name: Trileptal] (if unstable)

4) Primidone [Brand Name: Mysoline] (if unstable)
5) Phenobarbital (if unstable)
6) Bendamustine [Brand Name: Treanda]
7) Erlotinib [Brand Name: Tarceva]
8) Flecainide [Brand Name: Tambocor]
9) Irinotecan [Brand Name: Camptosar];

i) Vital signs outside of the following range (participants failing for vital signs will be allowed to re-screen once):
   1) Systolic BP greater than or equal to 160 mm/hg
   2) Diastolic BP greater than or equal to 100 mm/hg
   3) Systolic BP below 90 mm/hg and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
   4) Diastolic BP below 50 mm/hg and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
   5) Heart rate greater than or equal to 105 bpm
   6) Heart rate lower than 45 bpm and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint);
j) Expired air carbon monoxide (CO) level greater than 80 ppm;
k) Allergy to propylene glycol or vegetable glycerin;
l) Household member enrolled in the study concurrently;
m) Participated in prior study that involved reduced nicotine content cigarettes within the last 24 months;
n) Having participated in a research study during the past three months that would impact baseline smoking or response to study products;
o) Inability to independently read and comprehend the consent form and other written study materials and measures because participants are required to complete parts of the protocol at home independently;
p) Unstable living environment that would compromise the ability to attend visits, sequester study products to prevent use by other people or complete study procedures outside of visits.

9.3. Screening Procedure

Subjects will contact the clinics by telephone or through an online interest form. A telephone screening questionnaire will then be administered. If eligible based on initial screening, the subject will be invited to an Orientation visit to describe the protocol and then scheduled for the In–Person/Telehealth Screening visit (Orientation and In-Person/Telehealth Screen visits can be combined). Procedures conducted for screening are described in section 5.2.1. The data collected at this visit will be reviewed for further eligibility assessment and health forms will be reviewed by the medical professional to confirm stable physical and mental health.

9.4. Justification for Inclusion/Exclusion Criteria

Children under age 21 are excluded because they cannot legally buy tobacco products. Requirement of 5 cigarettes daily for the past month ensures changes will be observed in our

exposure biomarkers for the VLNC condition. Those subjects with unstable medical or medication conditions will be determined by the site's licensed medical professional and are excluded if these symptoms are believed to render the subject unable to fully participate in the study or pose a safety issue. Examples include but are not limited to the following: angina, stroke, heart attack, blood clots in the arms or legs for which the individual is undergoing active medical treatment, cancer requiring active chemotherapy or radiation therapy, severe arthritis, 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, suicidal ideation or any other condition which is likely to impair the individual's ability to attend and fully participate in study visits and procedures. Pregnant and nursing women are excluded as tobacco use is detrimental to the fetus or infant. Current or recent (3 month) alcohol or drug abuse problems will be excluded as these factors could independently affect smoking behavior and may affect the subject's participation in the study. Unstable living situations, such as homelessness, could compromise the subject's ability to control access to the investigational study cigarettes. Medical or psychiatric conditions (to be determined by medical history, CES-D, Prime-MD) may be deemed to pose a safety issue to the subject.

10. Vulnerable Populations

Vulnerable populations will not be recruited into this study.

11. Local Number of Participants

The total number of subjects to be randomized for this trial is 400-450. The intent is to recruit subjects across the six sites: University of Minnesota, Duke University, University of California, San Francisco, Wake Forest University, Brown University and University of Pennsylvania. If equal recruitment across sites is not possible, a site with faster recruitment may over recruit. Local and National Recruitment Methods

12. Recruitment Process

Once IRB approval is received and recruitment begins, cigarette smokers will contact the local study clinic in response to advertisements or word of mouth and receive information about the study. Interested potential subjects will be screened for initial eligibility over the telephone. If a site maintains a waitlist for potential subjects who have requested to be contacted if new studies begin, they will be called and screened for eligibility. If subjects meet the initial screening eligibility criteria for the study, they will be asked to attend an orientation meeting.

12.1. Source of Participants

Subjects will self-select into the screening process for the study. No medical chart review or direct patient solicitation will occur.

12.2. Identification of Participants

Self-identified subjects will contact the local research clinic in response to advertisements.

12.3. Recruitment Materials

Recruitment venues will include advertisement in local print media, internet (e.g. banner ads, Craigslist), social media (e.g. Facebook, snapchat, twitter), radio, television, and posted flyers and site's web page and waitlists. The verbiage in advertisements in print or verbal text is the following:

SMOKERS wanted for a study that provides research cigarettes with different nicotine levels and access to other tobacco, e-cigarette and vaping devices or nicotine products.
Joining the study is free and you will receive compensation for your time
Please call xxx-xxx-xxxx or go to [local website]
for more information and to see if you are eligible.
[local site institutional logo]

12.4. Compensation

Subjects will either receive cash, a gift card or a monthly check, depending on the site's institututional policies for providing compensation. Compensation includes $30 for screening, $40 per clinic visit for the participant's time ($520), $10 for travel ($140) for each of the 14 clinic visits, and an additional $15 for the 7 visits during which biomarker samples are collected ($105). After the follow up visit, they will receive a bonus for compliance with the study protocol requirements ($300 for avoidance of non-study combusted products; $100 for accurate self-report of slips using non-study products) and the amount earned for the daily call. The compensation for the completion of the daily calls will be $10 per week if they have not missed any calls ($160 total). The maximum earned will be $1,255 ($520 for visits; $140 for travel, $105 for first void sample collection; $300 for bonus; up to $160 for daily calls). (The maximum earned after converting to telehealth visits is $1240: $520 for visits; $130 for travel; $120 for biomarker collections; $300 for bonus; up to $170 for daily calls). They will also receive a voucher for a specified number of points per week (the amount is dependent on site and baseline smoking rate) to exchange for study products and at the end of the study will receive the amount that was not exchanged for products (1 point = $1 with a maximum of $200). Subjects must complete the study to be eligible to exchange their remaining vouchers at week 16 (unless withdrawn by PI for safety concerns).

13. Monitoring Safety and Withdrawal of Participants

13.1. Adverse Event Reporting and Safety Monitoring

Identifying Adverse Events

While participating in the trial, adverse events and concomitant medications will be assessed at every study visit and vital signs and carbon monoxide will be obtained. Adverse events typically will be identified during the administration of the Health Changes Questionnaire and Respiratory and Global Health Questionnaire, and in some cases during the administration of the CES-D. Other events may be identified from physiological study measures or by spontaneous reports during non-scheduled assessments. Withdrawal symptoms are considered an adverse event if the symptom had a significant impact on the participant's daily life, caused a major disruption of functioning, or took any medication for it.

Questionnaire items that will be reviewed:

- Health Changes Questionnaire: If the participant answers **'YES'** to **Questions 1, 2,** or **3** the interviewer should assess for adverse events.
  1) **Have you had any negative changes in your physical or mental health since your last visit?**
  2) **Have you received any form of medical care?**
  3) **Have you had any changes in medication since your last visit?**
- Respiratory and Global Health Questionnaire: If the participant indicates **'YES'** to **Question 6** regarding having a cold or flu, the interviewer should assess for adverse events
- CES-D: If the participant **scores 16 or higher and is not already being monitored for depression**, an Adverse Event for Elevated CES-D should be completed and the licensed medical professional will provide information regarding follow-up. If there is already an open event, information will be added to the existing form.

Physiological data that will be reviewed:

- CO level: The 'Adverse Event Log' should be completed if the average CO within a visit is:
  a. CO is greater than 50 ppm if CO at Baseline is < 20 ppm.
  b. CO is greater than 60 ppm if CO at Baseline is 20 – 34 ppm.
  c. CO is greater than 70 ppm if CO at Baseline 35 – 49 ppm.
  d. CO is greater than 80 ppm if CO at Baseline 50 – 64 ppm.
  e. CO is greater than 90 ppm if CO at Baseline 65 – 80 ppm.

- Blood Pressure:
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual blood pressure measurement during the same visit is **at or above 160 SBP or 100 DBP.**
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic and subsequent manual blood pressure measurement during the same visit is **below 90 SBP or 50 DSP and the participant is experiencing symptoms** listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'

- Heart Rate:
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' should be completed if an automatic **and** subsequent manual heart rate measurement during the same visit is **at or above 105 bpm or below 45 and symptomatic.**
- Since converting to telehealth visits, if vital signs are elevated during telehealth visits, the second reading will be conducted with the study supplied home monitoring device rather than a manual reading.

The site licensed medical professional will review all AEs. A study participant may be discontinued from the study if the medical professional and/or PI determine it is the best decision in order to protect the safety of a participant (see below). In the event that a participant either withdraws from the study or the investigator decides to discontinue a participant due to an AE/SAE, the participant will be followed, and if necessary referred to their physician, until the problem resolves, stabilizes, or is clearly unrelated to the study cigarettes. Any AE that remains open will be reviewed and closed at the 30-day follow-up interview.

13.2. Withdrawal of Subjects and Medical Monitoring

For the safety concerns, subjects will be withdrawn immediately from the study if any of the following occur:

1) Cardiovascular disease (CVD) event: Typically include*s* 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 subject will be withdrawn if he/she attempts suicide at any time during participation in the study.
4) Psychiatric Hospitalization: A subject will be withdrawn if he/she is hospitalized for psychiatric reasons at any time during participation in the study.
5) Pregnancy: If subject indicates she is pregnant or has a positive pregnancy test after receipt of study products, she will be withdrawn from the study if she is continuing to use any tobacco or ENDS product (oral medicinal nicotine use will be acceptable if approved by the LMP), and the pregnancy will be documented as an adverse event that 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 Adverse Event. A positive pregnancy test at Week 12 or follow-up will trigger an Adverse Event to be documented but will not result in withdrawal since she is no longer receiving study product.
6) Expired breath carbon monoxide increase: A subject will be withdrawn from the study if the average of the 2 CO readings is 100 ppm or greater.

The following will be monitored and can lead to withdrawal by the PI or licensed medical professional:

1) Blood pressure (BP) or heart rate (HR) changes: If any of the following occur post-enrollment: 1) BP is at or above 160 SBP or 100 DBP, 2) BP is below 90 SBP or 50 DBP **and the participant is experiencing symptoms** listed on the 'Blood Pressure and Heart Rate Symptom Checklist,' 3) HR is at or above 105 bpm, 4) or below 45 bpm **and the participant is experiencing symptoms** listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'

2) Expired breath Carbon Monoxide increase: An adverse event will be documented and the subject will be monitored by the medical professional if the average of the 2 (or 3) CO readings is:

    a. CO is greater than 50 ppm if CO at Baseline is < 20 ppm.
    b. CO is greater than 60 ppm if CO at Baseline is 20 – 34 ppm.
    c. CO is greater than 70 ppm if CO at Baseline 35 – 49 ppm.
    d. CO is greater than 80 ppm if CO at Baseline 50 – 64 ppm.
    e. CO is greater than 90 ppm if CO at Baseline 65 – 80 ppm.

3) Changes in tobacco product use: if during the weekly visits, participants' self-report a greater than 100% increase in cigarette per day (total cigarettes, including study and non-study cigarettes) relative to baseline, or a 50% increase in cigarettes per day relative to baseline that is accompanied by daily use of alternative nicotine products.

4) Medication changes: If a subject begins taking any of the exclusionary medications or other medications that could potentially have a smoking-drug interaction post-enrollment, the LMP will determine how best to monitor and minimize potential risks (including withdrawal if warranted). We will provide a letter to the participant that can be given to the prescribing physician and that lists potential medications that can be affected by changes in smoking that could occur as a result of participation in the study.

5) Any hospitalization or debilitation in which participation in the study could be detrimental to the recovery process. This will be self-reported by the subject and will be reviewed by the site PI and LMP to determine whether continued participation in the study is appropriate.

6) If a subject is behaving in an inappropriate or threatening manner, admits to lying about eligibility criteria, including omitting previous medical diagnoses and medications, is participating in other smoking research studies that could affect the primary outcome measures, does not follow study instructions, etc., then the PI can withdraw him/her from the study at the PI's discretion.

7) If a participant fails to attend his/her Randomization Visit within the 21-day allowable visit window, he/she may not be eligible to reschedule this visit or continue participation in the study without PI approval.

8) If there is reason to believe the participant is sharing large quantities of the study product with other people.

13.3. Withdrawal Procedures

If the decision to withdraw a subject occurs at a study visit, regular clinic visit procedures will be conducted as well as the additional termination procedures described in 13.4. If it is determined via a phone call that withdrawal is appropriate or if the subject is dropping out, we will ask the subject to attend an exit visit to return study product and equipment, complete questionnaires, product accountability and safety assessments and to receive their final study payment.

If the subject refuses an exit visit, we will request that at the minimum, study investigational product be returned and product accountability be completed. We will also stress the importance of being able to assess safety measures for the subject's safety.

13.4. Procedures for Early Termination Visits

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

A. Physiological Measures at Early Termination:

1) Expired breath CO level.
2) Vitals signs (blood pressure, heart rate).
3) Weight.
4) First morning void, (or if missing collect a spot urine) for biomarkers analysis.
5) Spot urine for pregnancy tests [hCG detection] for females (first morning void used at telehealth visits).
6) Spot urine sample for compliance testing (first morning void used at telehealth visits).

B. Questionnaires and Assessments at Early Termination:

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

1) Cigarette & Product Evaluation Scale – (Study Cigarettes and Usual Brand CES/PES)
2) CES-D:

   Beck Depression Inventory 2nd Edition (BD-II) will be administered on paper if the CES-D score is 16 or greater or if monitored for psychiatric illness.
3) Cigarette Dependence Measures (Study Cigarettes).
4) Vaping/Product Dependence Scale.
5) Environmental Tobacco Smoke Exposure Questionnaire.
6) Dietary Intake.
7) NIAAA Alcohol Use Questionnaire (1 month).

8) Perceived Health Risk of no tobacco use, usual brand cigarettes, study cigarettes, smokeless tobacco, medicinal nicotine and ENDS).
9) Cigarette/Product Purchase Task.
10) End of Study Questionnaire.

B.2 Administered as an interview by the study Research Assistant (entered into REDCap):

1) Health Changes Questionnaire.
2) Adverse Events.
3) Concomitant Medications.
4) Review of Daily Call and follow-up on missing and unusual reports.
5) Timeline Follow Back Questionnaire (since last visit).
6) Recreational Drug Use Questionnaire (1 month version).
7) Drug accountability: Complete study Product Dispensing Log.
8) End of Visit Questionnaire.

The data collected during the termination visit will be used as appropriate given the days outside of visit window, subject use of study product, measures of safety or other considerations to be determined in the data analysis.

14. Risks to Subjects

14.1. Study Related Risk

Potential risks of participation are minimal and include the following:

1) Survey Questionnaires: The interviews will include questions about medical history, drug and alcohol use, and questionnaires about mood. Answering these personal questions could make the subject feel uncomfortable.
2) Breach of Confidentiality: The risk of the interview is loss of privacy if other people find out the results from individual subjects.
3) Drug Testing: A breach of confidentiality could occur and other people could learn of the subject's drug use. (Discontinued since conversion to telehealth visits.)
4) Obtaining blood pressure: The blood pressure cuff may cause minimal discomfort. In obtaining blood pressure, researchers may find out the subject has abnormal blood pressure and must refer them to their primary care physician.
5) Smoking Cigarettes: All cigarettes are detrimental to a person's health and can lead to severe or fatal 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, tuberculosis and chronic airway obstruction

c. Cancers: Lung, bladder, liver, colon, cervical, esophageal, kidney, larynx, mouth, pancreatic, throat, stomach cancers and acute myeloid leukemia

d. Diabetes

e. Immune function, rheumatoid arthritis

f. Other Health Risks Associated with Smoking: Including but not limited to infertility, ectopic pregnancy, miscarriage, lower bone density in postmenopausal women, hip fracture in women, male sexual dysfunction; age-related macular degeneration, blindness and cataracts

6) Smoking VLNC Study Cigarettes (NNC are similar to commercial cigarettes): In addition to the above medical problems, subjects 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 or increase in the number of cigarettes smoked per day. This increased rate of smoking may persist after completing the study. 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. The study cigarettes are made from genetically modified tobacco plants. A recent toxicological analysis of these cigarettes showed that the concentrations of many harmful tobacco constituents, including polycyclic aromatic hydrocarbons, ammonia, and toxic metals, were similar to the concentrations of these constituents in commercial cigarettes [60].

7) ENDS: ENDS can expose users to several chemicals, including nicotine, carbonyl compounds, and volatile organic compounds, known to have adverse health effects. The health effects and potentially harmful doses of heated and aerosolized constituents of ENDS liquids, including solvents, flavorants, and toxicants, are not completely understood. ENDS aerosol is not harmless although it generally contains fewer toxicants and at lower levels than combustible tobacco products. Specific potential known risks include:

a. ENDS contain nicotine, which may contribute to some of the disease associated with smoking.

b. The most common side effects related to ENDS use are changes in taste, mucus in throat/sinus, dry mouth, dry cough, throat irritation, sore throat, mouth ulcers, dizziness, headache, and nausea.

c. While uncommon, the batteries from ENDS have exploded/ignited and injured users.

d. ENDS can overheat and present a minor burn risk if the button is turned on repeatedly. Subjects are told to be careful if storing the device in a place where the button might be accidentally pressed often. In rare cases the cartridge or pod containing the e-liquid has cracked or leaked e-liquid. Refilling of the tanks may also result in e-liquid spills. Subjects are instructed to immediately wash any exposed skin with soap and water.

e. Ingestion of ENDS liquids containing nicotine can cause acute toxicity and possible death if the contents of refill cartridges containing nicotine are consumed. Subjects are told to keep study ENDS and all e-liquid cartomizers away from pets and children.

f. Nicotine is an addictive chemical. It is possible that this experience could lead to long-term use of ENDS after the trial is over.

g. Allergic reactions to propylene glycol, vegetable glycerin or flavorants have been reported. If a subject reports a medical history of these allergies after randomization they will not be offered the vaping products in the marketplace. Chemicals used to flavor foods are also a risk for allergic reactions. We will ask subjects if they have had allergic reactions to specific nuts, fruits, and other flavors and advise them not to use e-liquids/cartridges that may contain or be labeled with those flavors.

h. Recent reports of seizure associated with vaping are being evaluated by the CDC and the FDA. While collection of evidence for seizure is not conclusive of causality, it is prudent to inform potential subjects of this investigation.

i. Reports of severe lung disease and deaths are being investigated by the CDC and the FDA. These incidents have been predominantly attributed to vaping THC and vitamin E acetate. Participants will be alerted to the symptoms of lung disease including: cough, shortness of breath, chest pain, nausea, vomiting, abdominal pain, fever. Participants are advised to discontinue use and promptly seek medical attention for any health concerns. Participants will also be advised to use only the study product we have provided to them and to not alter the device or add any substances to e-fluids. We will keep the participants informed as the additional information is provided by the CDC and FDA.

8) Use of Smokeless Tobacco or Snus: Smokeless tobacco products could increase risk for mouth sores, gum disease and tooth loss, nausea or upset stomach, vomiting, high blood pressure and nicotine addiction. Smokeless tobacco products may increase the risk of cancer, particularly oral, esophageal and pancreatic cancer. However, these risks are less than the health risks of smoking combustible cigarettes.

9) Use of Medicinal Nicotine (nicotine polacrilex and transdermal patch) or nicotine pouches: Most common adverse effects for medicinal nicotine include irregular heartbeat/palpitations, high blood pressure, mouth sores, mouth or throat irritation, heartburn, upset stomach, vomiting, diarrhea, dizzy or lightheadedness, and hiccups or belching. Additional adverse effects associated with nicotine gum include teeth or jaw problems. Additional adverse effects associated with nicotine patch are erythema, pruritus, edema and sleep disturbance. There may also be a risk of nicotine toxicity including symptoms such as nausea, dizziness, vomiting, diarrhea, and weakness.

10) Dual use of tobacco or nicotine products: There is also the chance of use of more than one product that may result in symptoms of nicotine toxicity and/or continued use of the products. However, cessation of all tobacco products will be strongly recommended to the subjects both at the end of the experimental phase and at follow-up with referrals to local cessation treatments or state quit line.

11) 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

12) 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.

13) 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.

14) Changes in Mood, Emotions and 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 use or cigarette consumption could adversely affect mood, emotions and the symptoms related to psychiatric conditions in some individuals.

15) Smoking and Oral Contraceptives in Women: Women who smoke and are over the age of 35 should not take oral contraceptives that contain estrogen without consulting their physician. Smoking while using oral contraceptives can increase the risk of having a cardiovascular event such as a heart attack or stroke. Additionally, there is a potential risk of thrombosis associated with hormonal therapy (including contraceptives) and smoking.

16) Smoking and Medications: Quitting smoking can greatly benefit participants' health. However, changes in smoking can lead to changes in how well some medications work. Participants are asked to disclose all medications they are taking. We also recommend that participants discuss any planned or actual changes in how much they smoke with their doctor, especially if they are taking any medications for psychiatric, cardiovascular, or other serious diseases.

14.2. Reproductive Risks

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. Smokeless tobacco/snus/nicotine pouches use increases risks for preterm delivery, preeclampsia or stillbirth and may have a negative effect on birth weight. Although unknown, similar effects could occur with the use of ENDS.

Avoiding Risks to Fetus

If female participants choose to be sexually active, they are asked to 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 should be using prescribed "birth control" pills, injections, or implants. Female participants with child-bearing potential will be tested for pregnancy at the first Marketplace visit and the randomization visit and monthly thereafter. If a participant becomes pregnant during the study, she will be withdrawn from the study if using tobacco or ENDS products. Approximately 30 days after being withdrawn or having a positive pregnancy test, 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.

14.3. Risks to Others

Tobacco products in the home can be toxic to children and pets. Smoking can lead to second hand exposure that can increase risk for disease. All participants will be informed to keep products not accessible to children and pets and informed about the harms of second hand smoke and vapor exposures in the home or in cars.

15. Potential 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.

16. Data Management

16.1. Data analysis plan

For complete statistical detail, please see Statistical Analysis Plan.

16.2. Randomization

Subjects will be randomized at the end of Phase 2 with a 1:1 ratio to the two studied conditions described above. Randomization will be stratified by site (6 study sites) using a blocked

randomization scheme with random block sizes of 2, 4, and 6. We anticipate no or less than minimal block effects in our data based on previous studies with similar design.

16.3. Sample Size

We will enroll 400-450 subjects (approximately 200-225 per group) to the study and assume a 20% attrition rate over the 12-week post randomization period. Power analysis was performed for two of the primary outcomes in the primary aim: CPD smoked at the end of Phase 3 and smoke-free days during Phase 3. The effect sizes and the distribution of population demographics were projected from a pilot study (U19CA157345; Hatsukami et al., 2017) and Donny et al. (2015)[16,14]. The two-sided type-I error rate for each outcome (CPD and smoke-free days) was set at α=0.025 (=0.05/2 outcomes by the Bonferroni method) for the overall sample. Powers for two independent subgroup analyses, by sex or by age, are also presented in Table 1, with type I error rate equal to α divided by the number of subgroups (=2) for each factor. T-test was used for the CPD outcome. For the smoke-free days outcome, we first calculated the rate of smoke-free days by dividing the number of smoke-free days by the total number of days in Phase 3 (approximately 84 days) and then used z-test for the Poisson rate difference. PASS 13 was used for power calculations.

Table 1: Power Analysis for Total Enrolled (randomized) N=450 and 400

Primary outcomes	VLNC	NNC	Overall sample	[3]Subgroup analysis by sex		[4]Subgroup analysis by age		
				F	M	18-24	25-44	45+
N enrolled = 450								
[1]Cigarettes (study or non-study) per day smoked at the end of Phase 3 (calculated as mean CPD based on 7 days' IVR data before the week 12 visit)	7	15	>.99	>.99	>.99	-	>.99	>.99
[2]Number of smoke-free days during Phase 3 (12 weeks or 84 days)	11 days difference		.92	.49	.56	-	.67	.37
N enrolled = 400								
[1]Cigarettes (study or non-study) per day smoked at the end of Phase 3 (calculated as mean CPD based on 7 days' IVR data before the week 12 visit)	Same as above		>.99	>.99	>.99	-	>.99	>.99
[2]Number of smoke-free days during Phase 3 (12 weeks or 84 days)			.88	.43	.50	-	.61	.32

[1]We assumed an equal SD for the two treatment arms. See Table 1 (left panel) in SAP for SD for the overall group, by gender and by age.

[2] The NNC arm's rate of abstinence was based on the interim analysis result (see Table 1 in SAP, right panel).

[3]44.7% female and 55.3% male, based on the interim data (see Table 1 in SAP).

[4]0.2%, 53.4%, and 44.7% for 18-24, 25-44, and >= 45 years old, respectively, based on the interim data (see Table 1 in SAP).

16.4. Study Populations

16.4.1. 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.

16.4.2. Definition of Sub-Group Population in Different Analyses

We intend to complete pre-planned subgroup analyses by sex, age groups (18-44, and 45+), and whether the participant was recruited pre- or post-COVID. Subgroup analysis will allow us to evaluate the consistency of the effect of VLNC vs. NNC across important subgroups.

16.5. Trial Endpoints

16.5.1. Primary Endpoints

- Cigarettes per day (CPD): the mean cigarettes (study and non-study cigarettes) smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit.
- Smoke-free days: the number of smoke-free days during Phase 3, based on IVR-Revised during Phase 3.

16.5.2. Secondary Endpoints

- Biomarker: percent change in biomarker, CEMA, at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Study Cigarettes per day (CPD): the mean study cigarettes smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit.
- Smoke-free point prevalence: Smoke-free days based on IVR-Revised and biochemical verification of smoke-free status (CO $\leq$ 6ppm) 7 days prior to the end of the 12 week.

16.5.3. Exploratory Endpoint

- Non-combusted tobacco/nicotine products use: the number of days using any non-combusted tobacco/nicotine products in Phase 3.
- Characteristics of products chosen (type, flavor, nicotine strength).

- Biomarkers: percent change in other biomarkers (Total Nicotine Equivalents, total NNAL, other mercapturic acids) at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Product satisfaction (Product Evaluation Scale if they used the product since the last visit; Cigarette Evaluation Scale if they smoked cigarettes since the last visit).
- Perceived health risk (Phase 1, Phase 2 and end of week 12 of Phase 3: all products for all subjects).
- Measures of discomfort/dysfunction: MNWS, QSU, CES-D, (NNC vs VLNC cigarettes irrespective of use of products).
- Dependence (FTND, WISDM, PATH items, monthly for study cigarettes)

16.5.4. Safety Endpoints

- Potential adverse consequences: Change in mental (CES-D) or physical health (heart rate, blood pressure, weight).
- Increased TNE (corrected by creatinine), calculated as change from Phase 1 baseline.
- Adverse events and Serious Adverse Events

16.6. Statistical Analysis

16.6.1. General Approach

All statistical analyses will be performed using SAS or R. All statistical tests will be two-tailed. A Bonferroni multiple-comparisons adjustment will be used to account for multiple comparisons for each of the two primary endpoints ($\alpha = 0.025$). Analyses of the secondary and exploratory endpoints will not be adjusted for multiple comparisons ($\alpha = 0.05$). All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below.

16.6.2. Describing the Study Population

Descriptive statistics will be performed for data collected at different visits. Daily Interactive Voice Response (IVR)-Revised data will be summarized by phase and by visit (according to the actual visit dates or scheduled visit dates if a visit is missed). Baseline data will be compared between the two studied conditions with t test or Wilcoxon rank sum test for continuous variables and chi-square test or Fisher's exact test for categorical variables, which include sex, age, race, education, menthol status, dependence (FTND), CPD, nicotine metabolite ratio (NMR) and TNE. Baseline CPD and TNE are based on Phase 2.

16.6.3. Primary Endpoint Analysis

The primary endpoints are (1) the mean cigarettes (study and non-study cigarettes) smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit; and (2) the number of smoke-free days out of the total number of days in Phase 3.

Primary Analysis

We expect the two groups to, on average, be balanced for important baseline characteristics due to randomization, so the primary analyses will be regressions for the difference in CPD and the number of smoke-free days between two conditions, adjusting for only the baseline value (the mean CPD and the percent of smoke-free days during Phase 2, respectively). Specifically, for the CPD outcome at week 12, we will use linear regression; for the number of smoke-free days, we will use Poisson regression or negative binomial regression when there is over-dispersion in the count data.

Secondary Analysis

For the Week 12 CPD and total number of smoke-free days, we will perform an adjusted analysis, adjusting for baseline value, study site (the randomization stratification factor), whether the Week 12 visit is pre-COVID or not, and any baseline characteristics (listed in Section 16.6.2), which are different at $p<0.20$, in multivariable regressions to improve efficiency.

For the CPD outcome measured repeatedly, we will use linear mixed effects models.[61] Fixed effects in the model will include: treatment group, visit, treatment group by visit interaction, baseline CPD, study site, whether the visit time is pre-COVID or not (as a time-dependent covariate), and any baseline characteristics (listed in Section 16.6.2) which are different at $p>0.20$. A random intercept for each subject will also be included in the model to account for within subject correlation.

16.6.4. Secondary Endpoint Analysis

The secondary endpoints will first be summarized by treatment group using mean and standard deviation. We will use similar adjusted regression methods for the secondary endpoints as for the primary endpoints described in Secondary Analysis Section. Details are described as follows.

- For the percent change (relative to the measure at the end of Phase 2) of the biomarkers measured repeatedly (weeks 4, 8, 12), we will use linear mixed effects models.[61] Fixed effects in the model will include: treatment group, visit, treatment group by visit interaction, and study site, whether the visit time is pre-COVID or not (as a time-dependent covariate), and any baseline characteristics (listed in Section 16.6.2) which are different at $p>0.20$. A random intercept for each subject will also be included in the model to account for within subject correlation.

- Study cigarettes per day will be similar to the Primary Endpoint analyses for total cigarettes per day.

- For the smoke-free point prevalence outcome, we will use logistic regression.

16.6.5. Exploratory Endpoint Analysis

We will conduct the following exploratory endpoint analysis:

- For the number of days of non-combusted tobacco/nicotine products use, we will use Poisson or negative binomial regression, adjusting for baseline value, study site, whether the visit is pre-COVID or not, and any baseline characteristics which are different at $p<0.20$.

- Descriptively examine the characteristics of the various products that are chosen (type, nicotine strength, flavor).

- Other biomarkers will be analyzed similar to the Secondary Endpoint analysis.

- Product satisfaction (Product Evaluation Scale, Cigarette Evaluation Scale) at the first time a participant tried a product will be summarized using mean and standard deviation and compared between two treatment arms using linear regression.

- Repeated measure of perceived health risk will be analyzed using the mixed effects model as described in the Secondary Analysis Section.

- Discomfort as assessed by MNWS-R, QSU, and CES-D will be compared between the VLNC vs. NNC cigarette conditions. The methods for comparing discomfort measures between VLNC and NNC cigarettes will be the same as for those described for the Primary Endpoints.

- Repeated measures of dependence of study cigarettes will be analyzed using mixed effects model described in the Secondary Analysis Section.

- Examine the cross-sectional associations of non-combusted products use with cigarettes smoked and biomarkers at different visits using marginal generalized estimating equations (GEE) model[62] (Chapter 12) with the non-combusted product use as the independent variable.

- We will explore baseline predictors of responses. We will use multivariable regressions to determine predictors of product use patterns, e.g., non-combusted product use, combusted product use, and smoke-free days. Predictor variables (depending on the responses measured) will include baseline CPD, total nicotine equivalents, FTND, age, sex, racial ethnic group, SES, perceived risk of products, product expectancy, product utility, social and environmental influences, contemplation ladder, menthol cigarette use and nicotine metabolite ratio (NMR, reflecting rate of nicotine metabolism). Similar regression models as described in Primary and Secondary Endpoint Analyses will be used.

16.6.6. Subgroup Analyses

Subgroup analyses will play an important role in understanding the effect of nicotine reduction for different subpopulations. We will explore the effect of VLNC vs. NNC within subgroups defined by age (18-44, and 45+) or sex. We will also conduct subgroup analysis by the time of the subject being recruited, pre- or post-COVID. Subgroup analysis will follow the same approach described above as for the overall sample, but significance will be assessed by a

Bonferroni-adjusted type-I rate (α/2 for a sex group or an age group, where α is the type-I error pre-specified for a specific outcome variable for the overall sample; see Section 16.6.1 for the values of α).

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

16.6.7. Safety

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

AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared across treatment groups. Count of AEs will be analyzed using Poisson regression or negative binomial regression as described in Secondary Endpoint Analysis.

16.6.8. 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-Revised as detailed in the study protocol (Compensation subsection 12.5). However, some level of missing data is inevitable in a study of this kind. We will compare subjects who do and do not complete the study in order to identify baseline covariates associated with the study completion.

We will use multiple imputation with the Markov Chain Monte Carlo (MCMC) method,[63,64] carried out in PROC MI in SAS. If the treatment group is associated with missing, we will conduct multiple imputation for each treatment group separately.[65] At least 10 imputed data will be generated, with the treatment effect being assessed in each imputed data set. A final single assessment of treatment arm difference will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS.

We will then conduct sensitivity analyses of primary and secondary endpoints by baseline imputation and the last observation carried forward (LOCF) method. Specifically, for the baseline imputation method, for a day with missing IVR-Revised data, the Phase 2 mean CPD will be imputed and non-abstinence will be assumed. For the outcome of biomarkers, missing data will be replaced by baseline values (i.e., no change in biomarkers). This will serve as a “worse-case scenario” as treatment group will be, on average, balanced at baseline. The

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

16.6.9. Interim Analyses

Interim analyses were conducted for re-estimation of sample size and will be conducted (blinded to investigators) for providing preliminary results to FDA if rulemaking for reducing nicotine in cigarettes to non-addictive or minimally addictive levels is proposed (see SAP for details).

16.7. Data Integrity

Adherence to protocol procedures, training and data integrity.

Standard operating procedures for all visits and procedures will be provided. Face-to face training for research personnel will occur at a site initiation visit prior to the study start, where the protocol and procedures will be carefully described. Case Report Form books will be created to maximize parallel recording of data across studies. Each visit will have a checklist of all the measures that need to be administered and the order by which these measures are administered. Additionally, conference calls will be held regularly with lead site, PIs and coordinators.

17. Confidentiality

17.1. Data Security

Subjects will be told their participation in the project will be strictly confidential, that any identifying information will be available to the project investigators or institutional or federal regulatory groups only, and that no identifying information concerning the data and results will otherwise be made known. Identifying information (initials and telephone numbers) entered in the Daily Call system will not be shared nor extracted with the data by the Biostatistics and Data Management Core.

Subjects will have written assurance that while de-identified individual subject data may be available to other internal researchers for research purposes, only a summary of the results will ever be published or otherwise publicly released. They will also be informed that all raw data will be coded with numbers and any form with identifying information (e.g., consent forms) will be kept separately in locked files. If subjects would like their information on vitals or other findings released to another party, they will be asked to sign a release form.

All electronic data will be de-identified, housed on the University of Minnesota AHC secured server. In–house access will be password protected. De-identified outcome data will be posted on a secure, password-protected website that is only available to research investigators (both

inside and outside the institutions affiliated with this U54 due to data sharing requirements; subjects will be informed of this during the informed consent process). All identifying information will be kept separate from the data in a locked cabinet in a secure place at the local site. Research study staff are required to complete site specific data security training through their institutions. There will be *no* data or consent forms will be placed in subject's medical records.

17.2. Certificate of Confidentiality

To help protect the participant's privacy, a Certificate of Confidentiality from the National Institutes of Health will be in place. 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 (e.g. guardian) 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 or elder abuse or a participant's threatened harm to self or others.

18. Provisions to Monitor the Data and Ensure Subject Safety

18.1. Data Integrity Monitoring

The Project Manager will oversee the day-to-day operation of the study. We will obtain IRB approval from all institutions prior to beginning the study. All personnel will be trained on study procedures, human protection issues and regulatory requirements. This will be conducted via an initial Start-up Meeting for all research Coordinators and site Principal Investigators. Prior to starting recruitment at the sties, an Initiation Visit will be conducted. At this visit, all research staff will attend to ensure that they possess adequate knowledge of the protocol and procedures. Standard Operating Procedures for all visit procedures, use of equipment, collection and entry of data will be reviewed at this visit. Any new staff will undergo training in these areas. To ensure correct data entry, outcome variable data will be double entered into REDCap and compared for discrepancies.

As another measure to ensure data collection integrity, the project manager from the University of Minnesota will make a visit to each site after they have started enrollment to make sure that the protocol is being followed carefully (and will include review of signed consent forms) and all the data is being collected properly. (The additional three sites (Wake, UPenn and Brown) did not have a site visit at the start of enrollment due to COVID-19 travel restrictions. Monthly quality checks monitoring the data and procedures were conducted through REDCap during this time.) Thereafter, these monitoring visits will occur every 12 months. (Annual monitoring visits were halted during COVID-19, however close-out visits will be conducted at all sites.) All subjective forms will be entered into REDCap including the telephone screening. Data on REDCap will be monitored remotely at the lead site (U of MN) for missing data or any unusual values or issues related to safety (adverse events and serious adverse events). This review will be provided to the respective sites in real time.

An external monitor through each of the institution's Clinical and Translational Science Award and/or the University of Minnesota Project Manager will review the study records to make sure that regulatory requirements are met. At this time, the consent forms will be reviewed (this would mean that the monitor will see the names of the participants; however no record of this identifying information will leave the premises), Delegation and Training Logs and IRB approval will be confirmed. On-site review of 5-10% of case report forms will be conducted unless there is cause for increased oversight.

The University of Minnesota NCI designated Cancer Center also has a Cancer Protocol Review Committee that meets on a monthly basis to review all cancer-related protocols. This study will be subjected to review by this committee. Progress reports to this committee are submitted on a yearly basis.

18.2. Data Safety Monitoring Plan

The DSMB for this project will begin by reviewing the protocol and establishing guidelines for data and safety monitoring. This will include developing standard procedures for day-to-day monitoring by the internal monitors, investigators and study staff. This Board will meet at regular intervals (at least once a year) to evaluate the progress of the trial, review data quality, patient recruitment, study retention, and examine other factors that may affect study outcome. They will also review the participant's ability to achieve the study requirements and the rates of adverse events to determine whether there has been any change in participant risk. Their review will ensure that subject risk does not outweigh the study benefits. A brief report will be generated from each of these meetings for the study record and forwarded to the Institutional Review Boards (IRB).

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

All product-related adverse events of a non-serious nature will be reported to the IRB at the time of renewal. Reportable serious adverse events will be submitted promptly. The DSMB will review all serious or unexpected adverse events and provide recommendations.

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

The study coordinator at each of the sites will be responsible for the daily oversight of subject safety. At each of the sites, the site PI and licensed medical professional (LMP) will meet regularly with the study staff to review patient's progress and their experiences with the tobacco products, including any adverse events. Entrance criteria will be reviewed following screening. Medical history will be reviewed by the LMP for any contraindications for the use of the study products and vital signs are checked at each clinic visit. Patients will be under medical supervision while in the study and seen on an ongoing basis by our research staff who will assess adverse events and make appropriate referrals to the LMP. The Data and Safety Monitoring Board (DSMB) and other regulatory bodies will be informed of any adverse events either at the regularly convened meetings or in the annual report, or if necessary, immediately.

19. Provisions to Protect the Privacy Interests of Participants

19.1. Protecting Privacy

Privacy will be maintained by meeting in a private room with subjects, they will be informed they may refuse to answer questions if they chose, but this may impact their eligibility. Subjects will be reminded that we have obtained a Certificate of Confidentiality to protect their data from being subpoenaed, particularly related to illegal drug use. Subjects are told that their identifiable information is not shared with any others outside of the research team.

19.2 Access to Subjects: There is no access to subjects' medical records or other private information.

20. Compensation for Research-Related Injury

There will not be any compensation for research related injury in this NIDA sponsored trial. The consent form will state: "In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study physicians know right away."

21. Informed Consent

21.1. Consent Process

Potential subjects will attend an orientation visit at the local clinic site where they will receive a copy of the consent form to read and be shown a standardized PowerPoint presentation showing the study purpose, procedures and responsibilities of participation. If the subject is to

return for the In-person/Teleheath Screening visit at a later date, they will take the consent home for review and signatures will be obtained at the In-person Screen. If the Orientation and In-Person/Telehealth Screen are combined, the process of obtaining informed consent will be obtained during Orientation and then the In-Person/Telehealth Screen will commence. Upon converting to Telehealth visits due to COVID-19 safety precautions, the orientation visit and PowerPoint presentation was conducted remotely and the consent forms were signed electronically.

The subjects will be solicited to ask questions and will receive answers. The coordinator/research assistant will then ask several open-ended questions to assess comprehension of the study prior to signing the consent form (What is the purpose of this study? Can you please describe the types of things you will be doing at the visits? What are some of the risks and discomforts you may experience while participating in the study? What are some of the benefits of being in the study? If you are eligible to participate, how many weeks will the study last?) After obtaining informed consent, collection of data can begin. The consenting process will be documented in the subject's case report form progress notes:

"Subject has read the consent form and the PowerPoint was presented for the study entitled "Impact of Very Low Nicotine Content Cigarettes in a Complex Marketplace." The study was explained in full detail and subject was allowed to ask questions and receive answers. Subject agreed to participate and signed the consent form on: _____and received a copy of the consent form."

If any changes in risk are identified during the course of the subject's participation, they will be informed of the issue and a new consent form will be presented and signed. The subject may choose to continue participation or withdraw from the study at that time.

21.2. Wavier or Alteration of Consent Process

None.

21.3. Non-English speakers/readers

Non-English speakers/readers are not eligible to participate.

21.4. Participants Who Are Not Yet Adults

Infants, children, and teenagers under 18/21 years of age (depending on site) are not eligible to participate.

21.5. Cognitively impaired adults

Cognitively impaired adults are not eligible to participate.

21.6. Adults unable to consent

Adults unable to consent will not be eligible to participate

22. Setting

Recruitment and visits will be conducted by research staff at the sites respective clinics. All original sites had an institutional IRB provide local approval of the study. For the new sites, Wake Forest University IRB will be the IRB of record and the original sites will seek reliance agreements to use this single site IRB

- University of Minnesota: Tobacco Research Programs: Delaware Clinic Research Unit at 717 Delaware St. SE, 200, Minneapolis, MN
- Duke University: Center for Addiction Science and Technology (CfAST) at 2608 Erwin Road, Suite 300, Durham, NC 27705
- University of California: UCSF Tobacco Research Center at 3130 20th Street Suite 308 San Francisco, CA 94110
- Wake Forest University: Tobacco Center of Excellence Biotech Place, 575 North Patterson Avenue, Suite 430, Winston-Salem, NC. 27101
- University of Pennsylvania: Center for Interdisciplinary Research on Nicotine Addiction (CIRNA), Perelman School of Medicine, University of Pennsylvania, 3535 Market Street, Suite 4100, Philadelphia, PA 19104
- Brown University: Center for Alcohol & Addiction Studies, Brown University School of Public Health, 121 South Main Street, Providence, RI 02903

23. Multi-Site Research

23.1. Study-wide Number of Subjects

Approximately 400-450 randomized.

23.2. Study-Wide Recruitment Methods

Similar methods of recruitment will be used across sites as described in Section 12.

23.3. Study-Wide Recruitment Materials

Similar recruitment materials will be used across sites as described in Section 12.

23.4. Communication Among Sites

Initially, weekly conference calls will occur among the research coordinators at each of the sites to track progress and trouble shoot and to make sure that all protocol procedures are followed. Approximately on a once a month basis, the PI and co-investigators will participate in this conference call. During these conference calls, the number of subjects enrolled, the data

collection process, the results from data monitoring and other issues of concern will be discussed. Once the study has been initiated and is running smoothly with fewer day to day issues to discuss, the research coordinator calls will be held bi-weekly and then monthly, unless there is cause for more frequency.

- As lead site, we will ensure that all sites have the current version of the protocol, consent form and HIPAA (if required) by providing these documents on our REDCap Administrative Projects site.
- IRB initial approvals and continuing reviews will be uploaded into REDCap Administrative Project site.
- Modifications to the protocol will be discussed on the Coordinator call. Submissions and approvals will be tracked on the REDCap website, which houses the site's currently approved protocol and consent form. Only after IRB approval will the site have access to download the newly approved consent form.
- All sites are academic institutions with the same rigorous requirements as the University of Minnesota's security provisions. Data will be entered into REDCap whose server is housed by the University of Minnesota's Academic Health Center. Identifiable HIPAA regulated information will not be shared between sites, all data entered is de-identified.
- All study sites have Federalwide Assurance and therefore have met the standards to comply with federal regulation and local laws:
    - University of Minnesota: FWA00000312
    - Duke University: FWA000009025
    - UCSF: FWA00000068
    - Wake Forest University: FWA00001732
    - University of Pennsylvania: FWA00004028
    - Brown University: FWA #00004460
- Reporting of Non-compliance:

  Any site noncompliance with federal regulation or state laws information will be provided to the University of MN, the lead site, and the offending site's IRB will be informed. UMN will provide this report to the UMN IRB.
- Reportable Events:
    - Reportable events will be reviewed in the GCP training session at the study start-up meeting to ensure site personnel is aware of the requirements. Any reportable events will be sent to the local IRB and the lead site (UMN). These include: death of subjects, new or increased risk, adverse events or safety reports that require a change to the protocol or consent form, deviations that affect risk, audits or inspections, written reports from sponsor or FDA, allegation of non-compliance, unauthorized disclosure of confidential information, unresolved subject complaint, suspension or premature termination of the study, incarceration of subjects, medical board or hospital staff actions, or any other information deemed related to research and puts subjects or other at increased risk of harm. In addition, adverse events that are unanticipated, related to the study and involving increased risk will be reported.
    - Serious adverse events (SAEs) as defined in 21 CFR 312.32 (death, life-threatening, hospitalization (initial or prolonged), disability or permanent damage,

congenital anomaly/birth defect, required intervention to prevent permanent impairment or damage (devices), other serious (important medical outcomes)) that are related or possibly related to study participation will be reported to the U54 Administrative Core, all site IRBs, the NIDA Scientific Officer (Ann Anderson, MD), the NIDA Project Officer (Kevin Walton, PhD), FDA, and the Data Safety and Monitoring Board. All Site IRBs require that fatalities related to the study be reported within 24 hours, that all other unanticipated product related SAEs be reported within 5 business days. Reports of all reportable SAEs will also be documented within FDA's reporting system, within 72 hours.

- Report of IRB actions: Actions taken by the local IRBs in response to SAEs will be reported to the lead site who will report to the FDA and NIDA as will reports of changes or amendments to the protocol as a result of an SAE. In addition, NIDA will be informed of any change that is significant enough for IRB review and changes the protocol in substantive ways (e.g., changes to procedures, recruitment strategy, inclusions/exclusion criteria), before such changes are implemented (except an urgent safety measure). Recommendation for trial discontinuation, for significant changes or amendments to the protocol, or other significant findings as a result of an SAE will be reported immediately to the FDA and NIDA Scientific Officer (Ann Anderson, MD) and Project Officer (Kevin Walton, PhD) by the Principal Investigator.

24. Resources Available

24.1. Feasibility of recruitment

A total of approximately 400-450 male and female subjects will be randomized in the clinical trial over the course of 4 years. Enrolling this number of subjects is not unreasonable. In a recently completed study, each of the sites enrolled around 125 subjects within 2 years. Four of the sites were also involved in successfully enrolling the required number of subjects for the Donny et. al. study.[14]

24.2. Staff Effort

Each site will have approximately 2 full time staff dedicated to this study, which will be sufficient for recruitment, conducting study visits and other procedures. Principal Investigators at each site will be devoting ~10% effort to the project. Each site has a Medical Director that is available for review of medical histories, adverse events or other issues involving medical oversight.

24.3. Facilities

University of Minnesota: The study will be conducted at the University of Minnesota's Tobacco Research Programs housed at the Delaware Clinical Research Unit at 717 Delaware St. SE Minneapolis, MN. Dr. Dorothy Hatsukami serves as the Director for this Program and Dr. Sharon Allen is the Medical Director. The shared space at the Delaware Clinical Research Units

includes waiting room with a receptionist, 7 physical exam rooms (two dedicated to the Tobacco Research Programs), 1 phlebotomy room, 5 interview rooms, 2 day-hospital rooms, an infusion room, 1 smoking laboratory with one way observation room, laboratory spaces for processing blood and urine, a locked medication supply room, locked protocol room for subject files, cubicles for data entry, management and analyses, locked supply storage and access to three conference rooms. Two restrooms are in the clinical space for urine collections. We have dedicated space, with key card access, containing ten -20 freezers. Equipment available includes vital sign and expired air CO and alcohol monitors. The study will also have access to all of the resources of the University of Minnesota for our use, as needed.

Duke University: The project activities will take place in the Center for Addiction Science and Technology (C*f*AST) founded and directed by Dr. Joseph McClernon (PI) and located in the Lakeview Plaza building adjacent to the Duke University and VA Medical Centers in Durham, NC. The PI has over 1300 square feet of laboratory space that includes two interview rooms, two physical exam rooms, a control room, three cognitive testing rooms, a scanner simulator room and a wet lab for conducting phlebotomy and biological specimen processing, and a dedicated sample storage room. In addition, the laboratory has two rooms equipped with a ventilation system that allows for indoor smoking. Finally, there is a secure room for medication storage and preparation. Equipment includes a computer system for electronic questionnaire administration (MediaLab), vital sign and expired air CO and alcohol monitors.

University of California-San Francisco: Research subjects are seen at the Tobacco Research Clinic located in San Francisco in close proximity to San Francisco General Hospital. The Clinic has a reception area and three private exam rooms for subject interviewing and specimen collection. The exam rooms are equipped with supplies to measure height, weight, blood pressure, expired CO, and breath alcohol levels. Additionally, two of the exam rooms have computers for administration of electronic questionnaires. There is a private bathroom for collection of urine samples. There is a lab where the specimens are prepared and equipment is housed. There are four separate rooms/offices where the clinical research coordinators and project manager are located and supplies are kept. A total of 9 separate computer stations are available for clinical research coordinator use. Additionally, the site has 2 laptops available for staff use. This is an off-site leased space located at 3130 –20th Street, Suite 380.

Wake Forest University: The study will be conducted at the Wake Forest Tobacco Control Center of Excellence housed at the Biotech Place in Winston-Salem, NC. Dr. Eric Donny serves as the Director for this Center. The space at the Wake Forest Tobacco Control Center of Excellence includes a waiting room with a receptionist, 7 ventilated testing rooms for smoking studies with audio and visual system for observation, 1 phlebotomy room, locked protocol room for subject files, cubicles and offices for data entry, management and analyses, locked laboratory space for product storage including 2 product refrigerators, 1 large wet laboratory space containing 2 -80 freezers and space for biosample processing, a conference room, and men’s and women’s restrooms adjacent to the suite for biosample collection. Equipment available includes vital sign and expired air CO and alcohol monitors. The study will also have access to all of the resources of the Wake Forest School of Medicine for our use, as needed.

Brown University: Research participants are seen at the Addictive Behaviors Research Laboratory at Center for Alcohol and Addiction Studies (CAAS), a 4800 square feet in size and was renovated per our specifications for the purpose of conducting human laboratory studies. The CAAS lab space contains 18 observation rooms, a room equipped for conducting physical examinations, a workroom for the storage and analysis of biological samples and a bathroom for participants' use. The CAAS lab is located in Brown University's Public Health building in downtown Providence, RI.

University of Pennsylvania: Research participants are seen at the Center for Interdisciplinary Research on Nicotine Addiction (CIRNA) Perelman School of Medicine at the University of Pennsylvania. The laboratory includes five 10x10 rooms equipped with exhaust fans and viewing windows to observe participants. Comfortable chairs placed in each. Restroom facilities are available for urine collection and there is a lab area for processing samples. Necessary equipment includes CO monitors, vitals monitors, laptop and desktop computers, and two refrigerators dedicated to specimen storage and two refrigerators dedicated to study product. The laboratory is located in Philadelphia.

24.4. Availability of Medical or Psychological Resources

Each site has a Medical Director and other study designated medical professionals, including access to a licensed psychologists that can be contacted if needed. Each site has specific Standard Operating Procedures that describe procedures to follow in case of emergency such as a suicidal participant. The medical staff will be trained in the study protocol, inclusion-exclusion criteria, and the conditions that may require temporary discontinuation of the study products or withdrawal from the study.

25. Conduct of the Study

25.1. Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

25.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the subjects. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

25.3. Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 7 years after the study file is closed with the IRB and FDA.

26. Subject Identifier

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

Project Identifier: F= CENIC 2 Project 1

Site Identifier:

B = University of Minnesota

C = Brown University

E = University of Pennsylvania

F = Duke University

H = University of California – San Francisco

M = Wake Forest University

Subject ID:

B001-300

C001–300

E001-300

F001-300

H001-300

M001-300

Data Collection Time Point Identification Numbers:

94= Screening Visit

93=Baseline Visit 1

92=Baseline Visit 2

91=Marketplace Visit 1

00=Marketplace Visit 2 and Randomization

01= Week 1 visit

02= Week 2 visit

03= Week 3 visit (discontinued in conversion to telehealth visits)

04= Week 4 visit

06= Week 6 visit

08= Week 8 visit

10= Week 10 visit

12= Week 12 End of Intervention visit

16= 30 day follow-up visit

Additional visit (AV1, AV2, etc.)

86 = Termination Visit

27. Procedure Table	Platform	SCRN 94	BL 93	BL 92	MP 91	MP 00	Week 1-3[3]	Week 4	Week 6	Week 8	Week 10	Week 12	Follow -Up 16	Early Term
			Phase 1		Phase 2		Phase 3							
Phone Recruitment Questionnaire	REDCap	X												
VISIT ASSESSMENTS														
Weight (Height at screen)	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test (if applicable)	Paper and REDCap			X		X		X		X		X		X
Heart Rate & BP	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
Cotinine check, if needed	Paper and REDCap	X												
Breath Alcohol Test[4]	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Toxicology[4]	Paper and REDCap	X										X		
Concomitant Medications	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
Health Changes Questionnaire	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events Assessment	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X
Product Accountability Log	Paper and REDCap			X	X	X	X	X	X	X	X	X		X
Procedures & Compliance Review	Paper			X	X	X	X	X	X	X	X	X		
End of Visit Evaluation	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
SCREENING QUESTIONNAIRES														
Tobacco Use History and Exposure & Cessation Methods	Paper and REDCap	X												
Identifying Information	Paper and Access	X												
Demographics for Registration	Paper and REDCap					X								
Demographics	EDC	X												
Brief Medical History	Paper and REDCap	X												
PHQ PrimeMD (Beck if applicable)	Paper and REDCap	X												
CES-D	EDC	X				X		X		X		X	X	X
Beck Depression Inventory/GAD (if + PHQ or Elevated CES-D)	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
(MINI) Suicide Subscale	On Paper only	X												
Drug Abuse Screening Test (DAST)	EDC	X												
Alcohol Use Questionnaire 12 mo	EDC	X												
Drug Use Questionnaire 12 mo	Paper and REDCap	X												
Michigan Alcohol Screening (SMAST)	EDC	X												
Assessment Name	Platform	SCRN 94	BL 93	BL 92	MP 91	MP 00	Week 1-3[3]	Week 4	Week 6	Week 8	Week 10	Week 12	F-Up 16	Early Term

Assessment Name	Platform	SCRN 94	BL 93	BL 92	MP 91	MP 00	Week 1-3[3]	Week 4	Week 6	Week 8	Week 10	Week 12	F-Up 16	Early Term
TOBACCO USE MEASURES														
Daily Call (IVR-tobacco diary)	IVR		X	X	X	X	X	X	X	X	X	X		X
Timeline Follow-back	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	X
IVR Review	Paper and REDCap		X	X	X	X	X	X	X	X	X	X	X	
ADVERSE CONSEQUENCES														
MNWS	EDCs		X	X	X	X	X	X	X	X	X	X		
QSU- Usual cigarettes	EDC		X	X	X	X								
QSU - Study cigarettes	EDC						X	X	X	X	X	X		
Alcohol Use Questionnaire 1 mo.	EDC		X			X		X		X		X		X
Drug Use Questionnaire - 1 month	Paper and REDCap		X			X		X		X		X		X
Respiratory & Global Health Quest.	EDC		X	X	X	X	X	X	X	X	X	X	X	X
DEPENDENCE MEASURES														
Cigarette Dependence Scale (FTND, PATH items, WISDM subscale)	EDC		X			X		X		X		X	X	X
Vaping/Product Dependence Scale	EDC											X	X	
Severson SLT Dependence Scale												X		X
Contemplation Ladder	EDC			X		X								
ACCEPTABILITY MEASURES														
Cigarette Eval. Scale Usual brand	EDC		X	X	X	X							X	X
Cigarette Eval. Scale Study cigs	EDC						X	X	X	X	X	X		X
Product Eval. Scale (all selected)	EDC				X	X	X	X	X	X	X	X		X
Perceived Health Risk Rating	EDC			X		X						X		X
Cigarette Purchase Task - Usual Cigs	EDC			X		X						X		X
Cigarette Purchase Task - Study Cigs	EDC											X		X
Product Purchase Task - All products	EDC			X		X						X		X
Assessment Name	Platform	SCRN 94	BL 93	BL 92	MP 91	MP 00	Week 1-3[3]	Week 4	Week 6	Week 8	Week 10	Week 12	F-Up 16	Early Term

Assessment Name	Platform	SCRN 94	BL 93	BL 92	MP 91	MP 00	Week 1-3[3]	Week 4	Week 6	Week 8	Week 10	Week 12	Follow -Up 16	Early Term
Biomarker Modifiers														
Dietary Intake	EDC		X			X		X		X		X		X
Environmental Tobacco Exposure	EDC		X			X		X		X		X		X
OTHER														
Social & Environmental Influences on Tobacco Use	EDC		X									X		
Product Expectancy Questionnaires	EDC			X										
Expected Utility (ANDS)			X											
End of Study Questionnaire	EDC												X	X
EXPOSURE BIOMARKER AND RISK FACTORS														
Carbon Monoxide	Paper and REDCap	X	X	X	X	X	X	X	X	X	X	X	X	X
Total Nicotine Equivalents (1st void urine)	BMW Database &Excel*		X	(X*)		X		X		X		X	X	X
Total NNAL (1st void urine)	BMW Database &Excel*		X	(X*)		X		X		X		X		X
Mercapturic acids of acrolein, benzene, crotonaldehyde, & acrylonitrile (1st void urine)	BMW e Database &Excel*		X	(X*)		X		X		X		X		X
8-epi-PGF (1st void urine)	BMW Database &Excel*		X	(X*)		X		X		X		X		X
Spot Urine or Saliva for compliance testing	BMW Database &Excel*						X	X	X	X	X	X		X
Nicotine Metabolite Ratio (saliva)	BMW Database &Excel*		X											
Genotyping (saliva)	BMW Database &Excel*			X										
Assessment Name	**Platform**	**SCRN 94**	**BL 93**	**BL 92**	**MP 91**	**MP 00**	**Week 1-3[3]**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**	**Follow -Up 16**	**Early Term**

[1] Administered if early termination is within visit window; [2] Samples may be used if subject was on study cigarettes at early termination visit; [3]Visit 3 was discontinued in the COVID-19 amended telehealth procedures; [4]Procedure was discontinued in the COVID-19 amended telehealth procedures* Banked and analyzed if needed.

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Statistical Analysis Plan

CENIC 2, Project 1

4/15/2017

Modified 1/30/2018

Modified 8/8/2019

Modified 10/16/2019

Modified 11/10/2021

Modified 4/11/2022

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Table of Contents

1. Introduction

This document will serve as the Statistical Analysis Plan for CENIC 2 Project 1, entitled "Impact of Very Low Nicotine Content Cigarettes in a Complex Marketplace". This document describes the planned statistical analysis for evaluating the impact of very low nicotine content cigarettes (VLNC) vs. normal nicotine content (NNC) in a complex marketplace. Details for the proposed analysis of the primary, secondary and other endpoints are provided.

2. Trial Objectives

The overall goal of this project is to determine pattern of product purchase and use when very low (0.4 mg/g) vs. normal nicotine (15.8 mg/g) content cigarettes are available in the marketplace. All participants will have access to non-combustibles (e.g., conventional smokeless tobacco, snus, e-cigarettes, medicinal nicotine) in addition to the study cigarettes, but no access to other combustible, non-study products (e.g., little cigars, cigarillos). The primary aim is to examine the effects of cigarettes that vary in nicotine content on the number of cigarettes smoked and days abstinent from combustible tobacco products in the context of a marketplace with alternative nicotine-containing products. The secondary aims are to examine the characteristics of products alternative to cigarettes that are chosen and to better understand responses to these various products.

3. Trial Design

This is a randomized, multi-center, non-blind study design. 700 subjects will be recruited from 3 study cites and randomized to one of two conditions:

1) very low nicotine content (VLNC) cigarettes with access to non-combusted tobacco/nicotine products; or 2) normal nicotine content (NNC) cigarettes with access to non-combusted tobacco/nicotine products. The two conditions will be referred as the VLNC and NNC groups, respectively, hereinafter.

Participants will undergo three experimental phases in the study: Phase 1: two weeks of baseline; Phase 2: two weeks of usual cigarette marketplace, where subjects will have access to their usual brand cigarettes and non-combusted tobacco/nicotine products; and Phase 3: 12 weeks' study cigarette market place, where subjects will have access to study cigarettes along with non-combusted tobacco/nicotine products.

3.1. Randomization

Subjects will be randomized at the end of Phase 2 with a 1:1 ratio to the two studied conditions described above. Randomization will be stratified by site using a blocked randomization scheme with random block sizes of 2, 4, and 6. We anticipate no or less than minimal block effects in our data based on previous studies with similar design.

3.2. Sample Size

Sample size was re-estimated for this project by Xianghua Luo, PhD on 8/21/2019. After discussion with members of the CENIC External Advisory Board and representatives of the National Institute of Drug Abuse and Food and Drug Administration, we changed the sample size from 700 to 500.

More power analysis was performed on 11/10/2021 for 2 smaller sample sizes (N = 450 and 400), with all the other parameters being the same as the power analysis performed on 8/21/2019.

We will enroll at least 400 rather than the 700 subjects originally proposed (200 per group) to the study and assume a 20% attrition rate over the 12-week post randomization period. Power analysis was performed for two of the primary outcomes in the primary aim: CPD smoked at the end of Phase 3 and smoke-free days during Phase 3.

3.2.1 Methods: See Section 6.9 for interim analysis and the methods for the sample size re-estimation.

3.2.2 Parameters based on interim data analysis

Table 1. Estimated Parameters Based on Interim Data

	Standard deviation (SD) of CPD of the pooled sample	Rate of abstinence of the control group
Overall	9.62	5.04%
By Gender		
Female (44.7%)	8.84	4.52%
Male (55.3%)	10.41	5.48%
By Age (years)		
18-24 (0.2%)	Not estimable due to the small sample size	No data
25-44 (53.4%)	10.20	2.85%
>=45 (44.7%)	8.95	8.04%

3.2.3 Power re-estimation

The power re-estimation is based on a total of 500, 450, and 400 enrolled subjects with a 20% attrition rate over the 12-week post randomization period. Power analysis was performed for the two primary outcomes: CPD smoked at the end of Phase 3 and smoke-free days during Phase 3. The effect of the two primary outcomes was assumed to be the same as previously assumed: 7 and 15 mean CPD for the VLNC and NNC group; and a 11 day difference (we assumed 12 abstinence days vs. 1 abstinence day, out of 84 days, previously) in the smoke-free days for VLNC vs. NNC. The nuisance parameters were based on the interim analysis (see Section 6.9 and Table 1 above).

The two-sided type-I error rate for each outcome (CPD and abstinence) was set at α=0.025 (=0.05/2 outcomes by the Bonferroni method) for the overall sample as previously designed. The type I error rate for the gender subgroup analysis was set to be α divided by the number of subgroups (=2) as previously designed. The type I error rate for the age subgroup analysis was set to be α divided by 2, rather than 3 because based on the interim data, there were <1% of participants in the 18-24 age group, hence, this age group was dropped from the power analysis. The Subgroup Analysis section (Section 6.6) in the SAP was revised accordingly.

The same statistical methods were used as previously designed. Specifically, t-test was used for the CPD outcome. For the smoke-free days outcome, we first calculated the rate of smoke-free days by dividing the number of smoke-free days by the total number of days in Phase 3 (84 days) and then used z-test for the Poisson rate difference. PASS 14 was used for power calculations.

The estimated power for 500, 450, and 400 subjects is presented in Table 2.

Table 2. Power Analysis for Total Enrolled N=500, 450, and 400

Primary outcomes	VLNC	NNC	Overall sample	[3]Subgroup analysis by sex		[4]Subgroup analysis by age		
				F	M	18-24	25-44	45+

N enrolled = 500								
[1]Cigarettes (study or non-study) per day smoked at the end of Phase 3 (calculated as mean CPD based on 7 days' IVR data before the week 12 visit)	7	15	>.99	>.99	>.99	-	>.99	>.99
[2]Number of smoke-free days during Phase 3 (12 weeks or 84 days)	11 days difference		.94	.55	.61	-	.72	.41
N enrolled = 450								
[1]Cigarettes (study or non-study) per day smoked at the end of Phase 3 (calculated as mean CPD based on 7 days' IVR data before the week 12 visit)	Same as above		>.99	>.99	>.99	-	>.99	>.99
[2]Number of smoke-free days during Phase 3 (12 weeks or 84 days)			.92	.49	.56	-	.67	.37
N enrolled = 400								
[1]Cigarettes (study or non-study) per day smoked at the end of Phase 3 (calculated as mean CPD based on 7 days' IVR data before the week 12 visit)	Same as above		>.99	>.99	>.99	-	>.99	>.99
[2]Number of smoke-free days during Phase 3 (12 weeks or 84 days)			.88	.43	.50	-	.61	.32

[1]We assumed an equal SD for the two treatment arms. See Table 1 (left panel) for SD for the overall group, by gender, and by age.
[2]The NNC arm's rate of abstinence was based on the interim analysis result (see Table 1, right panel).
[3]44.7% female and 55.3% male, based on the interim data (see Table 1).
[4]0.2%, 53.4%, and 44.7% for 18-24, 25-44, and >= 45 years old, respectively, based on the interim data (see Table 1).

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, age groups (18-44, and 45+), and whether the participant was recruited pre- or post-COVID. Subgroup analysis will allow us to evaluate the consistency of the effect of VLNC vs. NNC across important subgroups.

5. Trial Endpoints

5.1. Primary Endpoints

- Cigarettes per day (CPD): the mean cigarettes (study and non-study cigarettes) smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised (corrected with participant for errors) data before the week 12 visit.
- Smoke-free days during Phase 3: the number of days not smoking any combustible products based on IVR-Revised.

5.2. Secondary Endpoints

- Biomarker: percent change in biomarker, CEMA, at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Study cigarettes per day (CPD): the mean of study cigarettes smoked per day based on 7 days' Daily IVR-Revised data before the week 12 visit.
- Seven-day abstinence: if total CPD=0 for all 7 days before the week 12 visit and CO ≤ 6ppm at week 12.

5.3. Exploratory Endpoint

- Non-combusted tobacco/nicotine products use: the number of days using any non-combusted tobacco/nicotine products in Phase 3.
- Characteristics' of products chosen (type, flavor, nicotine strength).
- Biomarkers: percent change in biomarkers (Total Nicotine Equivalents, total NNAL, mercapturic acids at the weeks 4, 8 and 12 visits during Phase 3 as compared to the last visit in Phase 2.
- Product satisfaction (Product Evaluation Scale if they used the product since the last visit; Cigarette Evaluation Scale if they smoked cigarettes since the last visit).
- Perceived health risk (Phase 1, Phase 2 and end of week 12 of Phase 3: all products for all subjects).
- Measures of discomfort/dysfunction: MNWS-R, QSU, CESD (NNC vs VLNC cigarettes irrespective of use of products).
- Dependence (FTND, WISDM, PATH items; monthly for study cigarettes).

5.4. Safety Endpoints

- Potential adverse consequences: Change in mental (CESD) or physical health (heart rate, blood pressure, weight).
- Increased TNE (corrected by creatinine), calculated as change from Phase 1 baseline.
- Adverse events and Serious Adverse events.

6. Statistical Analysis

6.1. General Approach

All statistical analyses will be performed using SAS or R. All statistical tests will be two-tailed. A Bonferroni multiple-comparisons adjustment will be used to account for multiple comparisons for each of the two primary endpoints ($\alpha = 0.025$). Analyses of the secondary and exploratory endpoints will not be adjusted for multiple comparisons ($\alpha = 0.05$). All analyses will be completed using the intent-to-treat principle unless otherwise noted. Methods for handling missing data will be specified below.

6.2. Describing the Study Population

Descriptive statistics will be performed for data collected at different visits. Daily Interactive Voice Response (IVR) data will be summarized by phase and by visit (according to the actual visit dates or scheduled visit dates if a visit is missed). Baseline data will be compared between the two studied conditions with t test or Wilcoxon rank sum test

for continuous variables and chi-square test or Fisher's exact test for categorical variables, which include sex, age, race, education, menthol status, dependence (FTND), CPD, nicotine metabolite ratio (NMR), and TNE. Baseline CPD and TNE are based on Phase 2.

6.3. Primary Endpoint Analysis

The primary endpoints are (1) the mean cigarettes (study and non-study cigarettes) smoked per day based on 7 days' Daily Interactive Voice Response (IVR)-Revised data before the week 12 visit; and (2) the number of smoke-free days out of the total number of days in Phase 3.

6.3.1. Primary Analysis

We expect the two groups to, on average, be balanced for important baseline characteristics due to randomization, so the primary analyses will be regressions for the difference in CPD and the number of smoke-free days between two conditions, adjusting for only the baseline value (the mean CPD and the percent of smoke-free days during Phase 2, respectively). Specifically, for the CPD outcome at week 12, we will use linear regression; for the number of smoke-free days, we will use Poisson regression or negative binomial regression when there is over-dispersion in the count data.

6.3.2. Secondary Analysis

For the Week 12 CPD and total number of smoke-free days, we will perform an adjusted analysis, adjusting for baseline value, study site (the randomization stratification factor), whether the Week 12 visit is pre-COVID or not, and any baseline characteristics (listed in Section 6.2) which are different at $p<0.20$, in multivariable regressions to improve efficiency.

For the CPD outcome measured repeatedly, we will use linear mixed effects models (Verbeke and Molenberghs, 2000). Fixed effects in the model will include: treatment group, visit, treatment group by visit interaction, baseline CPD, study site, whether the visit time is pre-COVID or not (as a time-dependent covariate), and any baseline characteristics (listed in Section 6.2) which are different at $p<0.20$. A random intercept for each subject will also be included in the model to account for within subject correlation.

6.4. Secondary Endpoint Analysis

The secondary endpoints will first be summarized by treatment group using mean and standard deviation. We will use similar adjusted regression methods for the secondary endpoints as for the primary endpoints described in Section 6.3.2. Details are described as follows.

- For the percent change (relative to the measure at the end of Phase 2) of the biomarker measured repeatedly (weeks 4, 8, 12), we will use linear mixed effects models (Verbeke and Molenberghs, 2000). Fixed effects in the model will include: treatment group, visit, treatment group by visit interaction, study site, whether the visit time is pre-COVID or not (as a time-dependent covariate), and any baseline characteristics (listed in Section 6.2) which are different at $p<0.20$. A random intercept for each subject will also be included in the model to account for within subject correlation.

- For study cigarette smoked, similar analyses to total cigarettes per day will be performed.

- For the abstinence outcome, we will use logistic regression.

6.5. Exploratory Endpoint Analysis

- For the number of days of non-combusted tobacco/nicotine products use, we will use Poisson or negative binomial regression, adjusting for baseline value, study site, whether the visit is pre-COVID or not, and any baseline characteristics which are different at $p<0.20$.

- We will descriptively examine the characteristics of the various products that are chosen (type, nicotine strength, flavor).

- For other biomarkers, similar analyses to Secondary Endpoint will be performed.

- Product satisfaction (Product Evaluation Scale, Cigarette Evaluation Scale) at the first time a participant tried a product will be summarized using mean and standard deviation and compared between two treatment arms using linear regression.

- Repeated measures of perceived health risk will be analyzed using the mixed effects model as described in Section 6.3.2.

- Discomfort as assessed by MNWS-R, QSU, CESD will be compared between the VLNC vs. NNC cigarette conditions. The methods for comparing discomfort measures between VLNC and NNC cigarettes will be the same as for those described for the primary endpoints in Section 6.3.

- Repeated measures of dependence of study cigarettes will be analyzed using mixed effects model described in Section 6.3.2.

- We will examine the cross-sectional associations of non-combusted products use with cigarettes smoked and biomarkers at different visits using marginal generalized estimating equations (GEE) model (Diggle et al, 2002, Chapter 12) with the non-combusted product use as the independent variable.

- We will explore baseline predictors of responses. We will use multivariable regressions to determine predictors of product use patterns, e.g., non-combusted product use, combusted product use, and smoke-free days. Predictor variables (depending on the responses measured) will include baseline CPD, total nicotine equivalents, FTND, age, sex, racial ethnic group, SES, perceived risk of products, product expectancy, product utility, social and environmental influences, contemplation ladder, menthol cigarette use, and nicotine metabolite ratio (NMR, reflecting rate of nicotine metabolism). Similar regression models as described in Sections 6.3 and 6.4 will be used.

6.6. Subgroup Analyses

Subgroup analyses will play an important role in understanding the effect of nicotine reduction for different subpopulations. We will explore the effect of VLNC vs. NNC within subgroups defined by age (18-44 and 45+) or sex. We will also conduct subgroup analysis by the time of the participant being recruited, pre- or post-COVID. Subgroup analysis will follow the same approach described above as for the overall sample, but significance will be assessed by a Bonferroni-adjusted type-I rate ($\alpha/2$ for a sex group or an age group, where α is the type-I error pre-specified for a specific outcome variable for the overall sample; See Section 6.1 for the values of α).

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

6.7. Safety

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

AEs and SAEs will be recorded as described in the Adverse Event SOP. AEs and SAEs will be tabulated and compared across treatment groups. Count of AEs will be analyzed using Poisson regression or negative binomial regression as described in Section 6.4.

6.8. Missing Data

Every effort will be made to limit the amount of missing data in this trial. Study participants will be incentivized to attend study sessions and complete the daily IVR as detailed in the study protocol (Participate Compensation subsection). However, some level of missing data is inevitable in a study of this kind. We will compare subjects who do and do not complete the study in order to identify baseline covariates associated with study completion.

We will use multiple imputation with the Markov Chain Monte Carlo (MCMC) method (Schafer, 1997; Little and Rubin, 2002), carried out in PROC MI in SAS. If the treatment group is associated with missing values, we will conduct multiple imputation for each treatment group separately (Molenberghs and Kenward, 2007). At least 10 imputed data will be generated, with the treatment effect being assessed in each imputed data set. A final single assessment of treatment arm difference will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS.

We will then conduct sensitivity analyses of primary and secondary endpoints by baseline imputation and the last observation carried forward (LOCF) method. Specifically, for the baseline imputation method, for a day with missing IVR data, the Phase 2 mean CPD will be imputed and non-abstinence will be assumed. For the outcome of biomarkers, missing data will be replaced by baseline values (i.e., no change in biomarkers). This will serve as a "worse-case scenario" as treatment group will be, on average, balanced at baseline. The results of these analyses will be compared to the primary analysis to evaluate the robustness of our conclusions.

6.9. Interim Analyses

6.9.1. Interim Analysis for Sample Size Re-estimation (see above)

One interim analysis was conducted for sample size re-estimation. Nuisance parameters were estimated based on non-comparative data, whereas the significance level, the desired power, and the assumed targeted difference in outcome due to treatment assignment were not changed. In general, sample size re-estimations based on non-comparative data are expected to have a negligible effect on the Type I error (USDHHS, FDA, CDER, and CBER, 2018).

First, we calculated the distribution of gender and age (18-24, 25-44, and ≥45 years old). For the outcome of CPD, we calculated the variance for the pooled sample. For the outcome of cigarette abstinence, we evaluated the control (NNC) group's average rate of abstinence, which is calculated as the total number of smoke-free days observed among randomized participants divided by the total number of IVR days during Phase 3 (12 weeks).

6.9.2. Interim Analysis Blinded to Investigators

The purpose of this interim analysis is to provide the FDA with preliminary results from this study, if the FDA issues a Proposed Rulemaking for reducing nicotine in cigarettes to non-addictive or minimally addictive levels. This analysis will be conducted: (1) when the proposed rulemaking is issued; (2) by a statistician at Wake Forest who is independent of this project; (3) on the primary endpoints only. The results will be reviewed and submitted by a researcher who is independent of the project. Because this interim analysis will not be shared with the sponsor or investigators associated with the study, no changes will be made to the protocol as a result of this analysis.

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