

TRIAL PROTOCOL

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TITLE: The Effect of Very-Low Nicotine Content Cigarettes on Smoking in Non-Daily Smokers

Coordinating Center: University of Pittsburgh

Principal Investigator: Saul Shiffman, PhD
130 N. Bellefield Ave, Suite 510
Pittsburgh, PA 15213
412-567-1761
412-383-2041 (fax)
shiffman@pitt.edu

Statistician:

Brenda F. Kurland, PhD
201 N Craig St, Suite 325
Pittsburgh, PA 15213
412-383-1128

bfk10@pitt.edu

Study Coordinator:

Sarah Scholl, MPH
130 N. Bellefield Ave, Suite 510
Pittsburgh, PA 15213
412-383-2048
412-383-2041 (fax)
sarah.scholl@pitt.edu

Study Physician:

Esa Davis, MD
230 McKee Pl # 600
Pittsburgh, PA 15213
412-692-4862
davisem@upmc.edu

Responsible Data Manager:

James Moorehead
130 N. Bellefield Ave, Suite 510
Pittsburgh, PA 15213
412-383-2066
jdm166@pitt.edu

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NOTE: Protocol reflects study procedures at the time the first randomized clinical trial subject was enrolled (6/4/2015). Protocol changes from a pilot phase (such as the elimination of NNAL as a smoking biomarker, since we discovered that NNAL levels were often below the limit of detection of the assay) are not fully annotated. From the subjects' point of view, the only changes from the beginning of enrollment to the end of study were addition of a 30-day follow-up survey (which was not consistently implemented) and slight changes to the compensation structure.

PROTOCOL SUMMARY

In a two-arm study, 455 ITS will first be assessed for 2 weeks while smoking their own cigarettes, and then be randomized (double blind) to one of two matched research cigarettes (menthol if preferred): (1) VLNCCs (0.07 mg nicotine delivery) or (2) normal-nicotine content cigarettes (0.8 mg) for 10 weeks. Change in cigarette consumption (baseline to end of study) is the primary end-point, with 80% power to detect changes of +/-20% (after attrition). Cigarette consumption will be recorded in real time using an Interactive Voice Response (IVR) system, with complementary measurements collected at study visits (cigarette butts; Time-Line Follow-Back [TLFB] retrospective calendar-based self-report of research and non-research cigarettes smoked each day as well as any other sources of nicotine used since the prior visit).

Additional outcomes include urinary cotinine (nicotine’s major metabolite) and changes in per-cigarette smoking intensity (size and weight of cigarette butts, solanesol, a chemical measure of inhalation, and measures of smoking topography). Changes in cigarette satisfaction, craving, and nicotine dependence are also assessed, and the analysis explores potential moderators of the effects of VLNCCs, including race, baseline dependence, and history of prior daily smoking. Findings of this study will have important implications for the U.S. Food and Drug Administration’s (FDA) regulation of nicotine in cigarettes.

Summary of protocol modifications (excluding updates to personnel or to recruiting advertisements and methods) after opening enrollment to randomized clinical trial:

Date	Modifications
08/13/15	<ul style="list-style-type: none"> - Removed collection of medical information not directly related to the study, per advice of study physician - Added brief “screening-specific” consent form to allow participants to be screened for eligibility at the beginning of the session, without needing to be consented for the full set of study procedures (if they were to ultimately be found to be ineligible for enrollment) - Edited full study consent form to make language more concise - Added to Qualtrics phone screen a few questions to identify ineligible daily smokers at the beginning of the interview and thus make the process more efficient
11/19/15	<ul style="list-style-type: none"> - Adjusted the compensation structure, to distribute more evenly participants’ compensation across visits, rather than loading at the end of study. - Added a “drawing incentive” where participants receive a drawing ticket when they demonstrate excellent compliance and attendance. Tickets participants earn are placed into a bowl and one randomly chosen on a monthly basis. The participant whose ticket is drawn earns \$100.
08/11/16	<ul style="list-style-type: none"> - Added reimbursement for transportation expenses - Expanded eligibility criteria for participant age of eligibility – from 21 and over to 18 and over

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1. OBJECTIVES

1.1. To assess change (increase or decrease) in cigarette consumption from own-cigarette baseline over 10 weeks of smoking VLNCCs, compared to NNCCs. Cigarette consumption will be recorded in real time using an Interactive Voice Response (IVR) system, with complementary measurements collected at study visits (cigarette butts; Time-Line Follow-Back [TLFB] retrospective calendar-based self-report of research and non-research cigarettes smoked each day as well as any other sources of nicotine used since the prior visit). The study is powered to detect a + or - 20% change in consumption.

1.2. To use biomarkers and behavioral indicators to assess changes in per-cigarette smoking intensity:

- a. Urinary cotinine (a carcinogen and long-half-life biomarker) will assess overall exposure, and estimate changes in per-cigarette smoke exposure.
- b. Solanesol, a stable marker assayed in cigarette butts, will quantify smoking intensity in the field.
- c. Butt weight and length will quantify how much of each cigarette was smoked.
- d. Smoking topography measures will estimate changes in within-cigarette smoking behavior.

Additional exploratory aims include identifying individual differences that moderate response to VLNCCs, including (a) ethnicity (African American [AA] vs. white smokers), (b) history of past daily smoking and (c) starting level of dependence. We will also assess trajectory of changes in smoking behavior and changes in temporal smoking patterns, particularly examining possible increased concentration of smoking in bouts.

The project methods and measures are harmonized with Donny et al.'s research on VLNCCs in daily smokers,¹ and with FDA's national longitudinal PATH study of smoking behavior.² Findings of this study will have important implications for FDA's regulation of nicotine in cigarettes.

2. BACKGROUND

2.1 FDA regulatory authority over tobacco

The law granting FDA regulatory authority over tobacco mandates that FDA be guided by considerations of population health. In that framework, FDA is considering reducing (but not eliminating) the amount of nicotine in cigarettes.³ This follows a proposal by Benowitz and Henningfield⁴ that reducing the level of nicotine cigarettes deliver below the threshold necessary to sustain dependence (which was defined by reference to our early work on nondependent smokers⁵ would lead smokers to reduce their smoking or stop smoking altogether. Past versions of 'low-nicotine' cigarettes – so-called 'light' cigarettes – marketed by tobacco companies, appear to have actually increased smoking and toxicant exposure, because smokers who strive to maintain a certain nicotine level increased their smoking to compensate for the nominal reductions in nicotine yield.⁶ This was facilitated by the fact that the nominal reduced yield of the cigarettes was due to ventilation, which smokers could easily defeat to obtain more nicotine.^{7,8} A review of nicotine compensation with reduced-delivery cigarettes concluded that smokers typically increase their smoking to compensate for 50%-60% of the expected reduction in nicotine.⁹ The recent introduction of cigarettes with very low nicotine content (achieved through genetic engineering of tobacco⁹⁻¹¹) is meant to make compensation more difficult, as it would require substantial increases in cigarette consumption or smoking intensity to achieve compensation. Such Very-Low Nicotine Content Cigarettes (VLNCCs), with a range of yields below 0.1 mg nicotine per cigarette (compared to market average yield of 0.9 mg nicotine¹²), are seen as models for a potential FDA policy that would require all cigarettes to be VLNCCs.

2.2 Research on VLNCCs

A few small studies have tested VLNCCs in relatively heavy daily smokers (DS). These studies suggest that VLNCCs can maintain similar rates of cigarette consumption as NNCC cigarettes, suggesting they are still reinforcing,^{13,14} even though smokers prefer NNCCs.¹⁴ Compared to not smoking, VLNCCs increase ratings of satisfaction and liking,¹⁵⁻¹⁷ though less so than NNCCs,¹⁸⁻²⁰ suggesting they provide acute reinforcement. In DS, laboratory studies observe acute increases total smoke intake when smoking VLNCCs.²¹ The effects over longer periods are less clear: cigarette consumption was unchanged after a week of use,^{16,22,23} but declined over a 3-6 week period, as did nicotine dependence,²⁴ suggesting compensation may diminish with prolonged use (up to 6 weeks).^{16,22,24}

Based on these initial results, Dr. Eric Donny and colleagues undertook an FDA-funded, Pitt-IRB-approved, multi-center study to examine the effects of switching to VLNCCs of varying doses on cigarette consumption and exposures. This study randomized daily smokers (≥ 10 cigarettes per day) to cigarettes of varying nicotine levels for six weeks. Very-low-nicotine content cigarettes (VLNCCs) reduced average consumption by about five cigarettes a day, a decrease of 23-30%.¹

These findings support the potential FDA policy of capping nicotine delivery in all cigarettes, helping allay concern that such a change might actually increase exposure and harm. But, in order to make a complete and timely calculus of the public health effects of a reduced-nicotine policy, FDA will also need data on how it would affect ITS.

2.3 Non-daily Intermittent smokers (ITS)

It is not clear if existing findings on VLNCCs apply to non-daily smokers. The one study of VLNCCs in non-daily smokers found greater smoke intake from VLNCCs during an acute exposure.²⁵ No study has ever examined whether this compensation is maintained over time among ITS. Further, Donny et al.'s research excludes this 25%-33% of the US adult smoking population comprising 11-15 million US adults.²⁶⁻²⁹ This substantial group of smokers has been largely ignored in smoking research, and particularly in evaluation of nicotine policy and the effect of VLNCCs. Importantly, ITS do suffer severe health consequences from smoking: intermittent and very low-rate smoking is estimated to increase the risk of cardiovascular death by 40-60% (vs. nonsmokers).³⁰ A similar, if less dramatic, pattern is seen for risk of lung and gastrointestinal cancers: among very light smokers (up to 4 cigarettes per day; CPD) and ITS, risk of lung cancer is 2.8 that of non-smokers, while risk of stomach cancer is 2.4 times higher.³¹ While ITS are at lower risk than heavier daily smokers, they still suffer serious health consequences, and a substantial portion of ITS are vulnerable to progression to regular daily smoking, which poses even larger health risks.³²⁻³⁴ In our recent study of ITS, those whose FTND scores were anything but zero (about half) had a 4.5-fold increase in odds of progressing to daily smoking at 2 year follow up (results not yet published). Thus, ITS must be an integral part of tobacco control policy, including nicotine regulation.

2.4 ITS and nicotine

Although ITS are clearly not nicotine-dependent in the same way as DS, there is evidence that their smoking is motivated and maintained by nicotine. First, we have shown that they do take in the same amount of nicotine per cigarette as DS, and also metabolize it at the same rate.³⁵ This implies that they experience periodic spikes in nicotine levels, as shown and discussed below. It has also been shown that ITS are behaviorally affected by nicotine: In a carefully controlled study, normal-nicotine cigarettes (but not VLNCCs) acutely increased ITS alcohol consumption.³⁶ Further, our analysis of CPS³⁷ showed that many adopt nicotine replacement medication when they try to quit (albeit at even lower rates than DS);³⁸ we also saw this in a small pilot study of smoking cessation in ITS. Finally, a recent population-based study of 5,939 smokers found that ITS were more likely than DS to try, and – even more importantly – 3 times more likely to continue use of, ENDS (Electronic Nicotine Delivery Systems, or e-cigarettes), whose sole function is to deliver nicotine.³⁹ (58% of ITS who tried ENDS persisted.) This evidence, as a whole, suggests that ITS' smoking is at least partly motivated and maintained by nicotine.

It seems clear that ITS' relationship to smoking and nicotine is different from DS', and this may bear on how the two types of smokers might react to drastic reductions in nicotine delivery. A useful framework for describing the difference between DS and ITS was proposed by Russell, one of the seminal thinkers on smoking and nicotine.⁴⁰ Russell observed that most smokers (DS) are 'trough maintainers' who smoke to keep nicotine from dropping below a certain level, to avoid withdrawal – a hallmark of dependent smoking. This requires frequent, regular smoking, and is the smoking pattern upon which our models of smoking, dependence, compensation – and proposed nicotine-reduction policy – are based. But Russell observed that other smokers were 'peak-seekers' who did not try to maintain a minimum level of circulating nicotine but instead sought the acute nicotine effects achieved by spikes or peaks of nicotine. (Indeed, such acute effects are most readily achieved when smoking occurs against a background of abstinence: ongoing smoking tends to produce a persistent desensitization of nicotine receptors^{41,42}, resulting in fairly profound acute tolerance during ongoing smoking.)^{43,44} These considerations suggest how regular smoking leads to relatively stable nicotine levels throughout the day (trough maintenance), where intermittent smoking leads to distinct peaks, some of which represent substantial nicotine levels, achieved by smoking several cigarettes in close proximity (a "bout" of smoking). We believe that ITS are peak-seekers who smoke for the reinforcing acute effects of nicotine in particular settings that comprise discriminative stimuli for nicotine reinforcement.^{45,46} Unlike DS, who need to smoke continually to maintain a supra-threshold nicotine level, ITS smoke to achieve acute spikes in nicotine levels.

2.5 Effect of VLNCCs on ITS

If ITS smoke for nicotine, this implies they may also react to a radical change in nicotine delivery. It is possible that ITS will be deterred from smoking by VLNCCs – the acute effects of nicotine likely provide rapid feedback, such that cigarettes that did not deliver those effects would result in extinction. In this case, the salutary effects on ITS would enhance the beneficial effects expected in DS, further increasing the positive health impact of a VLNCC policy.

However, VLNCCs might have the opposite effect: increasing ITS smoking and exposure, and thereby causing harm to this substantial population of smokers. Indeed, one study already suggests that ITS actually do increase their smoking intensity in response to VLNCCs. Comparison of smoking topography when smoking cigarettes of high nicotine yield vs. smoking VLNCC cigarettes (0.07 mg nicotine) showed that adolescent non-daily smokers substantially increased their puffing and exposure (150%) when smoking the VLNCC cigarettes, achieving a remarkable 63% of perfect compensation for dramatically-reduced nicotine delivery.²⁵

Why might ITS show compensatory increases in smoking, even if DS do not? Because acute effects of nicotine offer rapid feedback, ITS may be able to immediately escalate their smoking to achieve the desired acute effects. Our preliminary data show that ITS already tend to concentrate their cigarettes in bouts: based on real-time recording of cigarettes via EMA, 72% of ITS recorded smoking bouts during their 3 weeks of EMA monitoring, and these ITS smoked over a third of their cigarettes in such bouts. Further, ITS have considerable ‘head-room’ to increase their smoking without incurring deterring toxicity. In our EMA study, we assessed experience of toxicity as a consequence of smoking. Cigarettes smoked in bouts were no more likely to make ITS feel sick than non-bout cigarettes, nor was there any increase in feeling sick as successive cigarettes were added to the bout. In other words, ITS can increase their smoking rate to at least that of DS without approaching any limit of subjectively-perceived toxicity. (DS likely have less ‘head-room’ to increase smoking, since their compensation would lead them to try to maintain levels throughout the day, and it would be hard to double or triple their already high smoking rates. In contrast, ITS trying to achieve an effective acute level in certain situations can increase substantially over their low baseline smoking rate.) Thus, ITS may react to VLNCCs by increasing their smoking, either by increasing the number of cigarettes or (as in the compensation study cited above) by increasing their smoking intensity (i.e., taking more or larger puffs). This would increase ITS exposure to tobacco toxins, and could increase, rather than decrease, harm to this substantial population. Yet, no study has examined the effect of switching to VLNCCs on ongoing smoking of ITS. Accordingly, it is imperative to evaluate the effect of VLNCCs in ITS, to inform FDA policy-making.

The proposed project would address a critical need in FDA’s exercise of regulatory authority. A nicotine-cap policy must be based on data regarding its public health impact on the entire population. A rationale for the policy that omitted consideration of 25%-33% (or more) of the US smoking population would be incomplete, and likely subject to challenge in the courts, particularly as it could have a differential adverse impact on ethnic minority smokers, who are more likely than whites to be ITS.⁴⁷ Establishing the effects of VLNCCs on ITS thus seems essential to inform FDA policy-making. If VLNCCs reduce ITS smoking, this would further support the public-health basis of such a policy. If VLNCCs increase ITS smoking, this needs to be taken into account in calculating the public health impact of the policy. Thus, evaluating the effect of VLNCCs on smoking ITS is crucial to informed FDA policy-making regarding the nicotine content of cigarettes.

While our proposed project does not study teen smokers or relatively new initiates, it may also have some relevance to those groups, as ITS smoking patterns in many ways resemble those of teens and young adults, both with regard to amount and frequency of smoking, but also with regard to situational patterns.^{48,49}

3. STUDY DESIGN

The proposed study is a 2-arm randomized experimental trial, with an own-control naturalistic baseline. The primary outcome is change in cigarette consumption, assessed by real-time self-report using Ecological Momentary Assessment, with additional endpoints of smoking topography and smoke exposure biomarkers.

The study has two phases. In the baseline (pre-randomization) phase, ITS continue to smoke their own cigarettes for two weeks, while cigarette consumption, topography, and biomarkers are assessed. For the second, intervention

phase, ITS are randomized, double blind, to switch to specially engineered research cigarettes with either very low nicotine content (VLNCC, 0.07 mg nicotine) or normal nicotine content (NNCC, 0.8 mg nicotine) for 10 weeks.

The NNCC group is needed to control for changes that might take place due to changing cigarettes (e.g., due to taste, brand switching) or to study procedures (monitoring, study visits), but not related to reduced nicotine content. Also, cigarette consumption can be affected by price.^{50,51} Since we are providing cigarettes for free during the experimental period, this could also lead to changes in smoking behavior not due to nicotine delivery per se. By collecting data on behavior with their own cigarettes, provided free, we are able to more clearly observe and attribute changes in behavior to the experimental manipulation of nicotine content. It should be noted that data we collected on ITS suggests that cost is NOT a primary factor holding down their cigarette consumption, suggesting that providing cigarettes for free should not significantly increase their smoking.

Both the baseline and experimental periods include ongoing assessment of cigarette consumption and periodic assessment of topography and biomarkers, along with assessment of craving, cigarette satisfaction and other subjective end-points. The primary analysis compares cigarette consumption in the NNCC and VLNCC groups in the final 2 weeks of the 10-week period of smoking the experimental cigarettes, compared to own-baseline smoking. Other key assessment points occur after the first 2 weeks of smoking experimental cigarettes, and near the mid-point of the experimental period, providing for assessment of the trajectory of changes as ITS adapt to VLNCCs.

4. SUBJECT SELECTION

4.1 Inclusion Criteria

- age 18 years or older (had been 21 years or older for part of the study period), and smoking cigarettes for at least 3 years (so that smoking is expected to be reasonably established)
- smoking, on average, 4-27 days per month (The lower limit avoids including ITS whose smoking is so sparse that it cannot be stably observed or assessed over a 2-week period; the upper limit avoids recruiting people who are essentially daily smokers.)
- smoking non-daily for the previous one year
- willingness to try novel cigarettes
- willingness and ability to come to the University of Pittsburgh's Smoking Research Group lab for 11 visits over a 12-week period, and to report on smoking behavior via an IVR telephone system during that time
- must be able to read and write English, since the study questionnaires are currently validated only for English-speaking populations

Note: There will be no constraints on the quantity of cigarettes participants typically smoke on smoking days (since this is often variable and is not a distinguishing feature of nondaily smokers).

4.2 Exclusion Criteria

- active plans to quit or actively seeking smoking cessation treatment in the next 3 months. (Subjects are permitted to quit during the study, and this will be assessed and analyzed, but those with stated active intention to quit at the time of screening will not be included.)
- severe psychiatric episode in the past month that may interfere with study procedures (though volunteers will not be otherwise screened for psychopathology or drug use, which are prevalent among smokers)
- current use of nicotine replacement or other tobacco products, by self-report in excess of 4 days per month (e-cigarette users will be excluded if their average monthly use exceeds half of their average monthly use of conventional tobacco cigarettes)
- exclusive use of roll-your-own cigarettes (since the study uses manufactured cigarettes); and cannot be currently smoking roll-your-own cigarettes on greater than 1/3 of smoking days
- [for female participants] being pregnant (by urine test) or breastfeeding, or planning to become pregnant
- current use of medications such as Chantix (varenicline), Zyban, Wellbutrin, or bupropion for any purpose, including stopping smoking
- myocardial infarction or cardiovascular condition occurring in the past month
- diagnosis of Buerger's disease
- night and/or 'swing' shift work (which complicates phone-based reporting system)
- known plans to relocate or move from the Pittsburgh area within the coming 3 months

- other household members are participating or have participated
- identification of cost being the primary reason why person does not smoke daily (so that the study outcome variable of smoking rate isn't influenced by participant receiving research cigarettes)
- at first session, reporting smoking 28 or more days of the past 30, or registering a CO reading of 15 or above.
- participation in more than 3 research studies in the past 6 months. Participation in a smoking study in the past 6 months. Participation in a study which involved medication within the last month

4.3 **Inclusion of women and minorities**

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.4 **Exclusion of pregnant women and women planning a pregnancy**

In order to protect against any potential risks to a fetus that can be caused by smoking during pregnancy, female participants will be screened for pregnancy via a urine-based pregnancy test prior to enrollment in the study, before any study cigarettes are distributed to them. They will also be advised during the consent process about the risks to a fetus should they smoke while pregnant, and as such, we will explicitly state the importance that they take steps not become pregnant should they decide to participate in the study. The consent form details methods to prevent pregnancy and advises participants that they will be withdrawn from the study should they become pregnant while participating.

5. **RECRUITMENT PROCEDURES**

Participants will be recruited from the surrounding community (greater Pittsburgh area) via multiple channels, including advertising and promotion (e.g., placards on public transportation advertising the potential study for which non-daily smokers interested in trying experimental research cigarettes may be eligible; ads on local radio stations); ads on television (using images approved for the placards on public transportation and a very similar format which was approved for Eric Donny's study: PRO11060292); by referral (from University-housed research registries); and social media outlets (e.g., Facebook, CraigsList, Twitter, YouTube). We plan on using YouTube as a way to display our television ad and will not be communicating with potential participants on it. We will only be posting study flyers on Facebook and Twitter and will not be communicating with potential participants. The flyers simply direct those interested in the study to contact us for more info.

We will also re-contact the 282 ITS who participated in our previous study (IRB0606147) and who consented to be re-contacted for other studies.

We will be using Pitt+Me (formerly called the CTSI Research Participant Registry) to advertise our study including social media (e.g., Facebook, Twitter).

We will also use the Text for Info system on our advertisements, SOSI (secure online subject interaction) from UPMC Department of Psychiatry's academic computing. This is where potential participants can text a number with a code word, which will reply to them more information about our study.

We will also be adding a website to our advertisements, smokingresearchgroup.com (please see: http://pitt.edu/~smokelab/srg_lab/ to see what it will look like), that contains basic information about the study and features a short web screen interested visitors can fill out.

We will also be giving bar coasters to local bars and restaurants.

Finally, in this study, as in our previous one, we will be assisted by Pitt's Center for Health Equity to help recruit AA smokers, using the Center's ties to the AA Community. The Center will help us to identify newspapers and other advertising venues that serve the AA community, to aid us in maximizing our reach in this community. Advertisements used for this purpose will be identical to those used elsewhere.

We will send a letter providing basic information about our study and how to contact us if interested (see up-loaded file) to potential participants, identified via review of medical records. The records will be extracted by the Center for Assistance in Research Using the eRecord (CARE), who will identify potential participants based upon their frequency and length of time smoking and age (18 or older). The data extracted will include the name, mailing address, age, and recorded smoking status of the potential participants. The extracted records will be transmitted via a restricted-access folder on the secure Sharepoint site called ReportHub. The medical records will not be retained beyond the period of active study recruitment.

6. REGISTRATION PROCEDURES

Participants will be recruited from multiple sources, as described above. Persons who express interest in learning more about the study via referral will be provided with the study contact phone number, which will also be included in all study advertisements. If potential participants are not able to reach a study research assistant directly, they will be asked to leave a message on our passcode-protected voicemail, so that study staff can later return their call. Once speaking with a potential participant, the study research assistant will ask the caller to identify as either a daily or non-daily smoker. Callers who identify as daily smokers will be given contact information of other local studies more appropriately suited to their smoking patterns. Callers who identify as non-daily smokers will then be asked about their interest in quitting. Non-daily smokers who express no interest in quitting will be read a brief description of the current study, and the research assistant will then ask if they would like to participate in a brief confidential phone screening interview in order to determine whether they may be eligible.

Potential participants will also be provided with a study website address from our advertisements. Potential participants will be able to access a short prescreen Qualtrics survey through this website. Going through this pre-screen potential participants will be able to identify as a daily or non-daily smoker, as well as answer a few additional questions about their smoking habits, which will help to determine whether they would be eligible for one of our studies (either this one or our other study for non-daily smokers, PRO13070454).

A waiver to document informed consent will be requested for the phone screening process and the online prescreen Qualtrics survey, since it presents no more than minimal risk of harm to subjects and involves no procedure for which written consent is normally required outside of the research context.

Individuals who are willing to participate in the phone screening will then be asked a series of questions to ascertain their eligibility. Importantly, participants who intend to quit smoking in the next 3 months are excluded from the protocol, so that procedures do not deflect them from quitting smoking.

Those who appear to meet the eligibility criteria based upon their answers during the phone screening will then be scheduled for an in-person appointment at the study site, at which time formal written consent to participate in the study procedures will be obtained. At this first in-person appointment, after the consent form is discussed and written consent to participate is obtained, a research assistant will review the phone screen questions with potential participants before officially enrolling the participant. The answers provided by the individual from the phone screen will be compared to the answers given in the in-person appointment to ensure consistency in self-report. Additionally, prior to enrollment, a research assistant will issue to the individual a calendar based TLFB, which captures smoking patterns over the prior 30 days, and if it is found that the participant has smoked on 28 or more of the past 30 days, they will not be eligible to participate. Finally, all participants will be asked to give a breath sample which tests for level of carbon monoxide (CO) present in the breath. Those participants whose CO level is greater than 15 will not be enrolled in the study (as this indicates heavy smoking and is not consistent with non-daily smoking.)

Also at the first in-person appointment at the study site, after the consent form is discussed and written consent to participate is obtained, female participants will be given a urine pregnancy test, as described in the screening interview and in the consent form, in order to verify the absence of pregnancy before formal study enrollment. Those who do not qualify due to having a positive pregnancy test or not meeting the other eligibility criteria will not be enrolled in the study. Should this be the case, we will issue \$10 in compensation for their time in coming to the lab.

Screening information from individuals who do not enroll will be asked if they would like to have their name and contact information retained in the study's secure screening database, so that they may be referred to other studies for which they may be eligible. Those who wish to do so will have consent documented. Otherwise, information

provided by non-participating individuals who do not wish to be contacted about future studies will be retained in aggregated form only, without identifying information, to be tabulated for the sole purpose of analyzing screening and recruitment statistics, and assessing potential volunteer bias. For subjects who are eligible and do volunteer, all information collected at screening will be retained at the study site for secure storage.

7. STUDY VISITS

7.1 Initiation Visit

At their first visit, potential participants will be told that the purpose of the study is to learn more about the impact that cigarettes with different amounts of nicotine have on smoking, and that the research may help inform the Food and Drug Administration on how best to regulate tobacco products in the future with the goal of improving public health, but that it is not a treatment program for quitting smoking. Potential participants will be told about screening procedures to determine their eligibility for the study and asked for consent for these screening procedures. For those eligible, research staff will explain all study procedures in detail and answer any questions potential participants have. Participants will be informed that if they choose to participate, they will be assigned by chance to one of two research cigarette groups, and will not be aware of which they are in. They will be informed that the research cigarettes they receive might contain less nicotine than what is found in conventional brands available for purchase in stores, including their own brand, and that they will be asked to smoke the research cigarettes (which will be either menthol or non-menthol according to their identified preference upon entry) exclusively over a 10 week period of the study. Consenting participants will complete an extensive questionnaire battery, and will be instructed to continue to smoke their preferred brand of cigarettes, which will be provided to them at no cost, as described above. Participants will be given cigarettes butt collection tins or bags and asked to retain butts from all cigarettes smoked, for submission at subsequent visits. Biomarkers (e.g., exhaled CO, urine specimen for analysis of cotinine and NNAL) will be collected, as will smoking topography measures (as described below). Participants will be trained on how to use the Interactive Voice Response (IVR) system to track their tobacco use (as described below).

7.2 Baseline

During the 2 weeks of baseline, participants will continue to smoke their own-brand cigarettes (provided gratis) ad lib, and to record each cigarette they smoke in real time using a simple IVR system (below), as well as collecting each cigarette butt (for counts and for biomarker assay). Participants will attend clinic visits weekly (Week 1 & 2) and complete the measures described below (Study Calendar).

7.3 Randomization Procedure

The random allocation sequence will be generated by automated software (sealedenvelope.com), operated by the PI, before any enrollment. Treatment group assignment is conveyed by color code to an administrative assistant (who is not involved in data collection), who prepares study cigarette packs. (See Section 8.2.) Correctness of assignment will be periodically audited by other staff not involved in data collection or subject interaction. Study subjects and all personnel involved in enrollment, data collection and recording, assessing outcomes, coding of adverse events, chemical assays, or data analysis are blinded to treatment group until after all subject visits are completed. Laboratories running chemical assays are blinded to treatment group assignment.

7.4 Experimental Period

At Visit 3, participants will be randomized (double-blind) to receive research cigarettes of either (a) normal nicotine content cigarettes (NNCCs; 0.8 mg/cigarette) or (b) very low nicotine content cigarettes (VLNCCs; 0.07 mg/cigarette). During the 10-week experimental phase, participants will attend 8 brief visits (weekly during the first 4 weeks, bi-weekly during weeks 5 – 8, and weekly at weeks 9 and 10). During these visits, IVR data and compliance issues will be reviewed, and participants will complete self-report and biomarker data collection. Topography measures will be collected at 3 of the 8 experimental period visits; see Study Calendar. Participants will continue to record smoking episodes via simple IVRs, and to collect and bring in during visits their cigarette butts and unused experimental cigarettes. For females, a pregnancy test will be conducted at visit 8 (approximately 6 weeks after randomization to receive either NNCCs or VLNCCs, in the experimental period).

7.5 Follow-Up

A month after the participant completes the study, they will be asked to complete a short online follow-up questionnaire.

7.6 Early Removal from Study

It is possible that subjects' participation may be discontinued from this study by the researchers at any time if it is determined that the participant is unable or unwilling to comply with the study procedures, or if it is discovered that the participant no longer meets criteria for inclusion in the study (e.g., participant found to smoke at a rate outside of those defined in our inclusion criteria).

Examples of inability or unwillingness to comply with the study procedures include: not showing up for multiple scheduled study appointments without having cancelled in advance; excessive cancellation of study appointments; demonstrated inability to follow the study's EMA protocol (i.e., not recording smoking occasions, not placing daily end-of-day calls, breaking or losing the study cell phone); behaving in an inappropriate or threatening manner during study visits; or demonstrating inability to comprehend the study procedures (i.e., severe cognitive impairment, coming to appointments under the influence of drugs and/or alcohol).

As a precaution for the participant's protection, participants will be monitored throughout the study for marked increases in smoking rate and/or in CO levels (measured at each study visit), and will be withdrawn immediately from the study if found to meet BOTH of the following two criteria at two consecutive study visits occurring during the experimental period (weeks 3 - 12, during which participants are smoking research cigarettes):

1) An increase in smoking, causing daily cigarette consumption to exceed twice their maximum number of pre-study-cigarette consumption (on days in which they do smoke), as observed during the two-week, own-brand baseline period, or as reported at entry into the study.

AND

2) Two consecutive expired breath carbon monoxide (CO) readings (during the same study visit) of:

- greater than 50 ppm if their CO value obtained at their baseline (first) study visit is <20 ppm;
- greater than 60 ppm if their CO at baseline is 20-34 ppm;
- greater than 70 ppm if their CO at baseline is 35-49 ppm;
- greater than 80 ppm if their CO at baseline is 50-64 ppm;
- greater than 90 ppm if their CO at baseline is 65-80 ppm.

[The above CO exclusion criteria mirror those used in Donny et al.'s protocol.]

8. STUDY AGENTS

8.1 Experimental Cigarettes

The experimental Spectrum-brand cigarettes used in the study (as in Donny et al.'s study, approved PRO11060292) will be supplied by the National Institute on Drug Abuse, which contracted their manufacture by XXII Century Tobacco. The experimental cigarettes are similar to the commercially-marketed Quest 3 cigarettes available prior to 2008 and were the basis of prior VLNCC research.^{16,24,52} These cigarettes differ in crucial ways from so-called 'light' or low-nicotine cigarettes marketed previously. Those typically had 'normal' amounts of nicotine in the tobacco rod, but diluted the smoke with air, with two consequences: the ventilation (tiny holes in the filter) was easy to defeat, making the actual nicotine delivery of the cigarettes quite malleable,⁵³⁻⁵⁶ and the nicotine and tar yields were highly correlated, as both were affected by ventilation.^{53,55,57,58} The XXII Century cigarettes are made from tobaccos genetically engineered to actually produce and contain different amounts of nicotine in the tobacco, so that (a) one cannot get more from them than they contain and (b) the nicotine content is manipulated independent of tar content. The cigarettes come in menthol and non-menthol varieties and are not branded.

The cigarettes come in a range of nicotine contents/deliveries. The 0.8 mg variety (In NIDA's designation: NRC 600/601) will be used for the NNCC condition; it matches the sales-weighted average smoking-machine-tested FTC

yield for the U.S.¹² (In our previous study of 282 ITS smokers, ITS brands averaged 0.7 mg, essentially equivalent.) The behavior of the NNCC control group will allow us to account for differences related to smoking new cigarettes (e.g., due to taste, brand switching) but not related to reduced nicotine content. For the VLNCC condition, cigarettes with 0.07 mg nicotine (NRC 200/201) – which is below the level where studies suggest compensation can be complete – are proposed.^{4,15,24}

The VLNCC and NNCC products differ systematically in nicotine content and yield (0.07 vs. 0.8 mg), but are matched on tar delivery, at 9 mg (the same amount that is delivered in standard, commercially-available cigarettes). Both are available in menthol (NRC x01) for smokers who prefer menthol.

8.2 Dispensing Cigarettes

Throughout the study, participants will receive their cigarettes from the study, gratis. During the baseline period, they will be offered cigarettes of their own preferred brand. (In our completed study of ITS, all ITS were able to identify a preferred brand, even if they were not completely brand-loyal.) This allows for assessment of baseline smoking (before switch to one of the two research cigarette conditions) on a common basis – i.e., without cost, but with monitoring in place.

At the start of the experimental period, participants will be randomized into either the NNC or VLNC cigarette condition. Participants will be supplied with cigarettes consistent with their randomly-assigned condition, matched to their own-brand menthol/non-menthol preference. Cigarettes will be dispensed in double blind fashion: a staff member not involved in contact with participants will be responsible for receiving research cigarettes from the Research Triangle Institute (RTI), labeling each carton with a blind code, and assigning product using this blind code based on the randomization schedule and participants stated menthol/non-menthol preference. The participants, investigators, and study staff will not know which type of cigarette is being dispensed.

To allow for adequacy of supply, in the event that a participant increases their expected cigarette consumption or misses a scheduled visit, participants will be provided at each visit a quantity of cigarettes equal to twice their mean reported consumption. To ensure product accountability, participants will be required to return at each visit any unused cigarettes and empty cigarette packs, as well as the butts of smoked cigarettes, and new cigarettes will be issued to replenish those used. Research staff will complete a product accountability log (again, modeled after Donny protocol PRO11060292) at each visit, and note any discrepancies between cigarettes dispensed and the quantity returned.

Participants will be instructed to smoke ad lib, but to smoke only the cigarettes issued to them and no others, and to refrain from other tobacco or nicotine products. However, participants will be told they will not be penalized for smoking or using other products and are asked to faithfully report such use. To enable participants to confine their smoking to the experimental cigarettes, participants will be instructed to carry the issued cigarettes with them when they might smoke. Most ITS already carry their own cigarettes: In our ITS study, subjects reported in real time where they got the cigarette they were smoking; only 7% never reported having their own cigarettes, and the remainder had cigarettes with them 83% of the time.

9. STUDY MEASURES

9.1 Measures of Cigarette Consumption

9.1.1. Ecological Momentary Assessment (EMA) via Interactive Voice Response (IVR) system:

EMA methods, developed and systematized by Dr. Shiffman,⁵⁹⁻⁶¹ focus on real-time collection of real-world data, in order to avoid biases introduced by recall and to provide temporal resolution. EMA methods are now widely used to collect data on smoking and other drug use,⁶²⁻⁶⁷ and we have demonstrated their successful use in multiple studies.⁶⁷⁻⁶⁹

In this protocol, given that our primary dependent measure is cigarette consumption, we will ask participants to record their smoking in real-time via a simple EMA method. Our approach, which we demonstrated successfully in a published study, imposes a very light burden on participants.

Participants will be trained to call an Interactive Voice Response (IVR) system that will register and record each call with a time-stamp, recognizing the caller on the basis of caller ID. Participants will be able to register multiple phone numbers, and if a call is received from an unrecognized phone number, the system will prompt the participant to enter their subject ID number. Participants will do this each time they smoke a cigarette, by pressing one button on their phone (programmed to auto-dial the IVR system telephone number). The calls will require no other interaction on the part of the participant – they will simply hear a message thanking them for recording their smoking, then the call will terminate. (Participants will also be given a “help line” number to call in the event that they dial the number by mistake, or need any assistance.)

In order to accurately collect data in real-time, participants must have access to and/or be willing to carry with them a cell phone during the study. Participants will be given the option to either: a) program the IVR system telephone number into their own cell phone or b) be provided by the study with a pre-paid cell phone they can use for this purpose. (2013 data show that 87% of US adults have a cell phone, and penetration is greater than 2/3rds even among those over 65 and those with less than high school education.⁷⁰ However, if participants do not have their own cell phone, have unreliable cell access, do not have enough monthly minutes in their own cell plan to afford to place the calls, or simply do not wish to use their own phone, they will be given the option of receiving a prepaid cell phone.)

In addition to providing accurate real-time records of cigarette consumption, this mode of data collection will provide information on temporal patterning of smoking (e.g., distinguishing cigarettes concentrated in bouts from those smoked at long intervals), and enable linkage between smoking occasions and the intensity at which cigarettes are smoked, data which solanesol analysis of cigarette butts will provide.

The IVR system will also be used to collect brief end-of-day reports during which, using the phone keypad, participants will report the total number of cigarettes they smoked that day (research and non-research), if any, that were not recorded in real time for any reason. The IVR system will also inquire about use of other tobacco and nicotine products each day, surveying all potential nicotine intake for the day, satisfaction with the cigarettes (validated Cigarette Evaluation Scale⁷¹), cigarette craving (from the Minnesota Withdrawal Scale⁷²), and nicotine toxicity symptoms.⁷¹ The end-of-day call is expected to last no more than 3 minutes in total length per day.

9.1.2. Time-Line Follow-Back:

Participants will also complete a calendar-based retrospective self-report of their smoking behavior and use of other sources of nicotine at each study visit. At the first visit, participants will report on the previous 28 days, and at subsequent visits, retrospectively of back to their previous visit. At each administration between visits, participants will indicate the number of research cigarettes smoked each day since the prior visit, as well as non-research cigarettes and use of any other tobacco or nicotine products. Participants will enter this information directly, using a tablet computer programmed to present input screens in the form of a calendar,

9.1.3. Cigarette butt count:

Participants will be asked to retain butts from all cigarettes smoked (including the ones that are not research cigarettes), by putting them into a specialized tin in the order they were smoked (skipping a space for omitted cigarettes), and submitting them at each visit. In the event that participants do not have the container given to them by research staff, they will be permitted to store butts in resealable plastic bags and/or plastic air-tight containers. The count of butts will represent an additional measure of cigarette consumption.

The multiple methods of estimating daily cigarette consumption (real-time IVR smoking-event entries, end-of-day IVR reports, and cigarette butt counts) will enable us to estimate compliance, and will provide multiple ‘views’ (ideally convergent) of cigarette consumption for analysis.

9.1.4. Behavioral assessments of per-cigarette smoking intensity:

Smoking topography will be assessed at visits 1, 3, 5, 8 & 11. Participants will smoke a single cigarette (of the type assigned at that time) through a cigarette holder using the CReSSMicro™ device, which measures multiple topography parameters (number of puffs, puff volume, puff duration, and inter-puff interval), yielding values that approximate measures in naturalistic settings. CO measures (below) will be taken immediately before and 15 minutes after the topography measure, at each visit in which topography is assessed. Since ITS usually smoke in particular contexts and may find it unnatural to smoke in a sterile laboratory environment, the topography room will be outfitted with comfortable furniture and décor to appear as a “smoking lounge.” (The Smoking Research Group

laboratory contains four rooms outfitted with external ventilation and has a waiver of no-smoking policies to allow smoking in lab.)

Cigarette butt length and weight. Cigarette butts collected from participants and brought to each visit by the participants will be measured and weighed to assess how much of the cigarette was smoked, another potential source of variation in per-cigarette exposure.

9.1.5. Biomarkers of exposure

9.1.5.1. Carbon monoxide (CO) breath sample:

CO in expired air will be assessed once at each of the 11 visits, and assessed a second time during visits 1, 3, 5, 8, & 11 (post-smoking topography assessment, per above). The CO measures will be collected using a Smokerlyzer ED50 CO meter (Bedfont Instruments) or Vitalograph-brand CO monitor. Breath sampling will take approximately 1 minute. Participants will be instructed to take three deep cleansing breaths, take a deep breath and hold it for 10-15 seconds, and then exhale completely into the collection tube. For each CO assessment, participants will be asked to follow the procedure twice, and the average of the two measures will be recorded. This is a reliable and valid measure of smoking, but is most sensitive to recent smoking, and is not entirely specific to smoking.

9.1.5.2. Urine collection:

Because breath CO sampling alone is not sufficient to verify smoking intensity, urine samples will be collected (and frozen) during at least 5 of the participant visits. Participants will be provided with a sterile collection cup, and will provide the sample in the lab's private restroom, outfitted with a stainless steel, medical grade specimen collection pass-through. All specimens collected will be transferred via pipette to polycarbonate storage vials, which will be labeled only with participant ID and collection date, and stored in locking freezers within the lab's secure suite. Four specimens will be sent to the University of Minnesota laboratory of Dr. Stephen Hecht for assay for cotinine, a nicotine exposure biomarker (analysis for NNAL was dropped after it proved to be insensitive and below the limit of detection in a substantial fraction of analyzed samples). (Dr. Hecht's lab is also doing the assays for the Donny et al. study of daily smokers, which will allow for some comparison.)

9.1.5.3. Solanesol in cigarette butts:

Solanesol is a stable alcohol found in tobacco leaf and deposited on the butt filter when cigarettes are smoked.⁷³ Its concentration in cigarette butts is a reliable and valid indicator of how much smoke was passed through the filter to the smoker. Watson showed that solanesol is an excellent linear marker for smoking intensity, as assessed by metrics such as number of puffs ($R^2=0.98$) and extraction of tar ($R^2=0.94$).⁷³

Participants will place their butts in resealable bags provided to them and return them to our lab. Solanesol is stable at room temperature for 4 weeks, allowing periodic collection of butts, which will be sent to Dr. Watson's laboratory at CDC (which is also performing solanesol analyses for Eric Donny's study of daily smokers) for analysis. The CDC lab will analyze 4 butts per participants at each of the designated outcome points in the study (weeks 2, 4, 8, and 12).

9.2. Other Assessments

9.2.1. One-time assessments at baseline:

All participants will be assessed via questionnaire measures during the first 3 laboratory visits. Questionnaires will be administered on computer tablets, using the questionnaire administration software Qualtrics, which enables automated data capture and implementation of intelligent skips and range and consistency checks, which minimizes errors.

In selecting measures, we have favored measures that are included either in Donny et al.'s protocol or in the FDA/NIH/NIDA PATH study,² a planned nationally representative, longitudinal annual survey of 59,000 people, whose measures (currently completing multi-site field-test) are likely to become gold standard measures in the field. (Dr. Shiffman is part of the Scientific Leadership Group designing this study.) This will allow for some comparisons of our results to others, and can help place our sample and results in a population context. PATH survey items are derived from a collection of standardized survey instruments in national surveys.

- Demographics. Basic demographic information (e.g. age, gender, ethnicity, education, income, occupation, marital status).
- Tobacco use history. Past and current smoking patterns (e.g. age at initiation, cigarettes per day, years smoked), brands smoked, interest in quitting, past quit efforts, difficulty quitting, etc.; current and past use of other nicotine (including ENDS) and/or tobacco products.
- History of daily smoking. Some ITS have a history of daily smoking, which appears to be an important source of variation among ITS, correlated with their dependence and smoking behavior⁷⁴ and also related to the likelihood that ITS progress to daily smoking (as assessed in our follow-up study). Past daily smoking may be an important moderator of ITS response to VLNCCs. We will also assess parametric details: how heavily ITS smoked when smoking daily, for how long, and how long ago.
- Past-month Smoking Pattern. The calendar-based TLFB^{75,76} will capture participants' smoking pattern over the previous 30 days preceding the first laboratory visit.
- Smoking among the participant's social network. Relevant items from the PATH and BRFSS, to assess potential influence of participants' social networks on smoking behavior.
- Medical History and Status. Relevant medical history and current health status, including any pre-existing diagnoses, symptoms and health problems that may be related to smoking.
- Perceived Health Risk. Perceived risks associated with smoking, both regarding their usual brand and VLNCCs, based upon items from the HINTS questionnaire.

9.2.2. Assessments at baseline and each 'outcome' visit:

- Nicotine dependence measures: Participants will complete several measures of nicotine dependence, including: 1) the Fagerström Test for Nicotine Dependence,⁷⁷ 2) the Hooked On Nicotine Checklist,⁷⁸ a measure often used to detect nascent signs of nicotine dependence, 3) the Nicotine Dependence Syndrome Scale,⁷⁹ and 4) an 8-item abbreviated version of the Wisconsin Inventory of Smoking Dependence Motives-68⁸⁰ developed for FDA's national PATH survey. This multi-faceted approach to assessing dependence will provide a more nuanced profile of dependence and smoking motives across participants, and may provide important insight into specific features of that may predict behavior change in response to VLNCCs. Importantly, our study of ITS showed that despite the generally low level of dependence, there is meaningful variation in nicotine dependence among ITS.⁷⁴ Baseline nicotine dependence may be an important moderator of ITS response to VLNCCs. Change in dependence can also be regarded as an outcome, in that VLNCCs may reduce dependence in those who had some degree of dependence at baseline, or potentially increase it in those who did not.
- Alcohol use quantity/frequency. Since ITS smoking is associated with alcohol drinking,⁴⁹ quantity/frequency of alcohol consumption will be collected.
- General Health and Respiratory Symptoms. Self-reported respiratory symptoms (e.g., cough, phlegm production, shortness of breath, etc.) will be collected using the Respiratory Health Questionnaire,¹ which may pick up short-term effects of reduced or increased smoking.
- Health Status, Medications, and Adverse Events. Medications will be recorded on a Concomitant Medications Questionnaire and participants will be asked about changes in health status. Adverse events will be solicited and recorded in the open-ended manner typical of clinical drug trials. The study physician will review adverse event reports and material changes in health status. All serious, unexpected, and apparently related adverse events will be reported to the IRB.

9.2.3. Exit interview

We will be giving an exit interview questionnaire to participants on their last visit.

10. ADVERSE EVENT MONITORING AND REPORTING

All subjects will be asked about any problems experienced during each regular study visit and instructed on how to reach the study staff or investigators, to report immediately any unexpected or serious adverse effects. In the event of a serious adverse event, Dr. Shiffman and/or Dr. Davis will contact the participant and the IRB according to the timelines and guidelines for the University of Pittsburgh. If an adverse or serious event occurs and neither Dr. Shiffman nor Dr. Davis can be reached, the participant will be instructed to dial 911 for immediate medical assistance and/or to report to the nearest emergency room. No events related to study procedures are expected.

10.1 **Potential risks from smoking research cigarettes:**

Randomized studies with daily smokers have uniformly shown that use of VLNCCs reduce smoking and promote quitting. However, it is possible that ITS assigned to this condition might react differently and, at least temporarily, increase smoking. Chronic smoking produces numerous health risks to the individual; however, the individuals being recruited smoke at very low rates (estimated at 4.39 cigarettes per day on 4 days of each week, less than 20 per WEEK, based on our previous study of ITS). It is possible that they may increase their smoking temporarily if assigned to smoke VLNCCs (this is part of what is being tested in the study), but the increase is likely small and short-lived. Importantly, the period during which participants are exposed to experimental cigarettes is limited to 10 weeks. National data (CPS)³⁷ show that the average ITS has been smoking for over 20 years, making it unlikely that this small, brief (and unlikely) increase in exposure will materially increase their risk.

To ensure that increases in cigarette consumption are limited, participants will be monitored throughout the study for marked increases in smoking rate and/or in CO levels (measured at each study visit). Participants reporting marked increases in smoking rate and CO level (as described subsequently) will be withdrawn immediately from the study as a precaution to minimize any potential risks to participants.

Nevertheless, participants will be fully informed of the risks to smoking in general during the consent process. They will also be informed of the potential to develop temporary withdrawal symptoms should they be assigned to receive very-low-nicotine-content cigarettes. However, given that nondaily smokers by definition regularly and repeatedly abstain from smoking (i.e., they do not maintain consistent levels of nicotine in their body), and have been demonstrated in our previous study not to suffer withdrawal when they abstain, it is considered unlikely that this population of smokers will experience withdrawal symptoms.

All participants will be counseled to quit smoking at the end of the study, and offered materials and referrals to assist them in doing so.

11. STUDY CALENDAR (shaded visits are longer)

# of weeks of study participation	Visit #	Cigarettes provided	Phone Reporting	Questionnaires given	CO Test	Butts collected	Urine collected	NNAL, Cotinine, Solanesol	Smoking topography	Pregnancy test	Compensation provided		Bonus
											Participation	Transportation	
0	1	Own-brand		X	X		X		X	X	\$20	\$5	
1	2	Own-brand	X	X	X	X					\$20	\$5	
2	3	Research	X	X	X	X	X	X	X		\$20	\$5	\$10
3	4	Research	X		X	X					\$20	\$5	
4	5	Research	X	X	X	X	X	X	X		\$20	\$5	\$10
5	6	Research	X		X	X					\$20	\$5	
6	7	Research	X		X	X					\$20	\$5	\$10
7	No visit	---	---	---	---	---	---	---	---	---	---		
8	8	Research	X	X	X	X	X	X	X	X	\$30	\$5	\$20
9	No visit	---	---	---	---	---	---	---	---	---	---		
10	9	Research	X		X	X					\$30	\$5	\$20
11	10	Research	X		X	X					\$40	\$5	\$10
12	11	None (study complete)	X	X	X	X	X	X	X		\$60	\$5	\$20

In addition to the above, participants who demonstrated full compliance with study procedures and who attended all study visits received a ticket to be entered into a drawing. Each month, one ticket was chosen at random from among those participants earning a ticket in that month, and participant whose ticket was drawn received a \$100 bonus upon completion of the study.

12. DATA REPORTING / REGULATORY CONSIDERATIONS

12.1 Data Management

Almost all data are collected electronically on validated systems. Database systems will be used to track timely receipt of data, and track biomarker samples through storage, shipment, and assay. (We will use the same systems as Donny et al.)¹ Data will be backed up and archived regularly, and edits will maintain an audit trail. Our lab has extensive experience with large and complex datasets drawing on multiple data sources.

12.2 Data Safety and Monitoring

The principal investigator, Dr. Saul Shiffman, will be responsible for monitoring the safety of this study and implementing a data and safety monitoring plan. In this, he will be assisted by Dr. Esa Davis, an internal medicine physician. Together they will ensure that there are no changes in the risk/benefit ratio during the course of the study and that the confidentiality of research data is maintained. In addition, a Data and Safety Monitoring Board (DSMB), comprised of John Hughes, MD (chair), University of Vermont; Jonathan Foulds PhD, Penn State University, College of Medicine; Peter Callas, PhD, University of Vermont; and Matthew Carpenter, Medical University of South Carolina, all experts in the field of smoking research, will be utilized to evaluate and oversee the data safety and monitoring plan for this project.

Each member of the study team will meet with the study coordinator, who will ensure that all personnel have completed required human subjects protection training prior to having contact with research participants or working with study data. Study personnel will meet weekly with the PI to discuss the study and to address any issues or concerns that have arisen. Minutes will be kept for these meetings and will be maintained in the study binder.

All procedures will be monitored to ensure that they conform to the approved protocol. In addition, monitoring will be done of all unforeseen circumstances that might arise and affect safety; of all reports of serious adverse events as (death, new or prolonged hospitalization, persistent or significant disability/incapacity, or congenital anomaly/birth defect); of other significant adverse events (adverse events that lead to drop out by the participant or termination by the investigator); of unexpected adverse events resulting from the study, and of expected adverse events.

Any adverse events will be reported immediately to the University of Pittsburgh IRB using the procedures stated in the IRB reference manual. The yearly IRB renewal for this study will include a summary report of the Data and Safety Monitoring Board findings from the prior year.

13. STATISTICAL CONSIDERATIONS

13.1 Primary Analysis Plan

For regulatory purposes, it is important to provide transparent, easily-interpreted analyses without complex statistical models. Therefore, the primary endpoint is the change in weekly cigarette consumption from the 2-week own-cigarette baseline to the final 2 weeks of the intervention period. (The final two weeks should best represent the lasting impact of the intervention.) While cigarette counts from TLFB data on ITS show a skewed distribution, change scores approximate a normal distribution. Therefore, group differences (VLNCC vs. NNCC groups) may be estimated using linear regression with 2-sided Wald tests and $p < 0.05$ as the threshold for statistical significance. Person-level covariates (e.g., stratification variables, demographics) will be used in regression models to explain additional variation in consumption changes. The primary analysis will provide point estimates and 95% confidence intervals for average change in consumption for each treatment group and for the group difference. Analyses will be weighted to rebalance the intentional oversampling of AA smokers.

13.2 Analysis Plan: Secondary Endpoints and Additional Analyses

Missing data and dropout are inevitably concerns in randomized trials. Because study visits are relatively frequent, data collected during visits is likely to be informative about the causes of subsequent dropout. Therefore, we will use linear mixed effects models to take advantage of implicit imputation of data missing at random. Measures of cigarettes smoked, smoke exposure, and smoking intensity can be modeled longitudinally for the intervention period, with baseline measures as covariates. Linear contrasts for fixed effects can be used to estimate a treatment

effect for weeks 11-12 (the final two weeks of the intervention period), while incorporating data from other time-points and covariates, and accommodating missing outcome data via use of random effects models. For participants who drop out of the study intervention, efforts will be made to conduct an end-of-study visit and to record reason for dropout (e.g., dissatisfaction with study cigarettes, versus the burden of participating in study visits), so these can be modeled. (We note that Dr. Kurland, the study statistician, is an expert in modeling and handling of missing data in randomized trials.) Of course, we will aim to prevent or minimize missing data. The study team has a strong record of recruitment and retention, including with ITS.

Additional study endpoints may be addressed using similar analysis methods as for the primary endpoint. Composite measures of total exposure will be computed by multiplying per-cigarette exposure measures (e.g., solanesol levels, total puff volume from topography) by total cigarette consumption for the baseline and end-of-study periods, and comparing change across groups. If a substantial proportion of participants achieve cessation (abstinence during the 2-week end-point assessment period(s)), the primary analysis may need to address over dispersion, and/or examine treatment group comparisons in cessation status (logistic regression) or in time-to-cessation (survival models). If dropout due to cigarette dissatisfaction and “cheating” (using commercial cigarettes without dropping out) are prevalent in either or both treatment groups, these behaviors may be examined as secondary endpoints, again using either logistic regression or survival models. These behaviors can also be incorporated into multivariate analyses modeling the main outcomes. Additional end-points such as craving and dependence can be modeled as outcomes, and also analyzed for their correlation with changes in cigarette consumption, and as possible mediators of reduction (or lack thereof) and drop-out.

Random effects models can also address effects over the entire experimental period, producing area-under-the-curve type estimates of changes in cigarette consumption over the study period. Methods that consider the patterns of change over time, such as trajectory mixture analyses⁸¹ can shed light on different trajectories or patterns of smoking behavior change during the intervention (e.g., identifying those that change immediately and persistently, those who change more slowly or transiently, and those who don’t change at all), which can then be related to known participant characteristics to identify potential moderators of change.

Subgroup and effect modifier (moderator) analyses. Although they will be underpowered, exploratory subgroup analyses will be conducted. First, effect modification will be examined: the treatment effect may differ by degree of dependence (assessed by FTND and PATH-WISDM at baseline), ethnicity (AA vs. White), or history of daily smoking. Other analyses will evaluate hypothesized mechanisms for observed treatment effects and explore covariates thought to be in causal pathways related to smoking behavior. For example, predictors of cessation could be examined within the VLNCC group. Analyses will follow the same principles as for the primary analyses described above, using models appropriate to the distributions of covariates and outcomes.

13.3 Sample Size Calculations

Based on prior Time-Line-Follow-Back data, the average weekly cigarette consumption is 21.5 (+ or - 18.2) cigarettes, with 0.70 within-subject correlation between two-week periods (corresponding to our baseline and end-of-study endpoints). For the purpose of sample size calculations, we assume no change in weekly NNCC consumption. Using nQuery Advisor 7.0 to estimate power for a between-group difference (baseline to week 12) equal to 4.3 cigarettes/week (20%) change in consumption indicated 80% power to detect that effect with an evaluable sample of 364 (182/group). Similar power was obtained using a simulation approach with 10,000 simulated datasets analyzed by univariate linear regression, and under a scenario of heterogeneous response where 40% of VLNC participants did not change smoking behavior, 40% decreased by about 4.3 cigarettes/week, and 10% achieved cessation. Since attrition is not accounted for in this simplified analysis, the targeted enrollment is 455 to account for a 20% dropout rate.

SECTION 2: METHODS

Non-daily smokers' changes in cigarette consumption with very-low-nicotine-content cigarettes: A randomized double-blind clinical trial

SECTION 2: METHODS TABLE OF CONTENTS

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1. SAFETY MONITORING

Safety monitoring was conducted by three approaches:

- 1) The Respiratory Health Questionnaire¹ was completed at each in-person study visit. Events such as flu, allergies, nausea, and cough were included in the mid-study DSMB report.
- 2) Adverse events were solicited and recorded in the open-ended manner typical of clinical drug trials. The study physician reviewed adverse event reports and material changes in health status. Adverse events reported pre-randomization and post-randomization were described in the mid-study DSMB report.
- 3) Reported CPD and exhaled CO were monitored for dramatic increase from baseline tobacco exposure (Protocol section 7.6).

Despite that tobacco exposure risks in smokers are unlikely to be affected by a 10-week change in cigarette smoked, safety monitoring was emphasized in the protocol. This emphasis was motivated by concern that subjects could increase smoking substantially during study participation, either because cigarettes were free or to overcome low nicotine content of very-low-nicotine-content cigarettes.

2. STATISTICAL CONSIDERATIONS

2.1. Analysis sets

The primary analysis was conducted in all randomized subjects (intention-to-treat, ITT). The dropout rate was lower than anticipated, and multiple imputation has gained acceptance in the medical literature, so the ITT population was selected over the “completers” analysis reflected by power calculations (Protocol section 13.3).

Unblinding occurred when all randomized subjects had completed the end-of-study follow-up (phone contact 30 days after study completion) or been characterized as lost to follow-up. The analysis database was locked to preserve subsequent data cleaning and coding reflected in the submitted manuscript.

2.2. Primary endpoint (cigarette consumption): Computation and multiple imputation

Cigarettes per day (CPD) was averaged over two-week periods to emphasize overall exposure over periodic consumption variation. Agreement among the two self-reports (Interactive Voice Response (IVR) by phone; Timeline Follow-back (TLFB)⁷⁶ recall questionnaire) and butt counts was found to be excellent in a pre-unblinding

analysis⁸², so these measures were averaged to increase precision.

All three cigarette consumption measures were summarized (and averaged) at the daily level, so uniform two-week intervals from randomization could be maintained even if subjects' study visits were not timed precisely. Dates with missing data (as opposed to days with no cigarettes smoked, as with an empty bag returned for butts) were excluded from CPD calculations for each measure, and two-week periods with fewer than 5 days of data were scored as missing. (With 5+ days the incomplete report was seen as preferable to imputation.)

Although the dropout rate for this study (as for others conducted by this group) was moderate, dropout was likely associated with cigarette consumption. Therefore, the primary analysis was performed on datasets with multiple imputation of CPD data missing due to dropout. Multiple imputation for monotone missing data was conducted by fitting a regression model from observed data, and drawing from the posterior predictive distribution of regression parameters to generate imputed values, as described by Rubin⁸³ and implemented in SAS PROC MI.⁸⁴ The "missing at random" assumption appeared justifiable because observed measures were fairly good at predicting the modest amount of dropout. Initial multiple imputation models followed the practice of inclusion,⁸⁵ with several baseline measures, component CPD elements (IVR, TLFB, butt collection), and compliance variables hypothesized to be associated with dropout. However, these models were prone to producing imputed values outside the range of the data, and the CPD elements had much worse agreement than for observed data. A more parsimonious imputation model for CPD composite measures was generated, with 25 imputation datasets to ensure reliable inference.⁸⁶

Variables in the CPD imputation model were:

- Age
- Gender
- Treatment group
- Education (8 levels)
- Race (White/African-American/Other)
- Annual income (midpoint of 12 categories, \$2,500 - \$90,000)
- Number of years as a smoker
- Maximum number of cigarettes smoked on any day in the last 2 months
- Past daily smoker vs. native ITS
- Total Nicotine Dependence Syndrome Scale (NDSS)⁷⁹ Score
- Wisconsin Inventory of Smoking Dependence Motives (WISDM)⁸⁰ Primary Dependence Motives Scale + Tolerance Item
- exhaled breath carbon monoxide concentration from screening visit (ppm)
- Days per week smoked during 2 weeks pre-enrollment (from TLFB)
- CPD on days smoked during 2 weeks pre-enrollment (from TLFB)
- Composite CPD (TLFB, EMA, butt collection daily CPD average), averaged for 6 biweekly periods:
 - Baseline (pre-randomization, subject's own cigarettes provided gratis)
 - Post-randomization weeks 1-2
 - Post-randomization weeks 3-4
 - Post-randomization weeks 5-6
 - Post-randomization weeks 7-8
 - Post-randomization weeks 9-10

Notes: In fitted MI models, the fraction missing CPD information based on variance ranged from 0.4% (weeks 1-2) to 5.2% (weeks 7-8 post-randomization). Only a few baseline values (mostly income) had missing values; these were imputed (single imputation) with other baseline demographic variables and dependence measures prior to multiple imputation of CPD.

Variables in the baseline imputation model were:

- Age
- Gender
- Treatment group
- Education (8 levels)
- Race (White/African-American/Other)

- Annual income (midpoint of 12 categories, \$2500 - \$90,000)
- Number of years as a smoker
- Maximum number of cigarettes smoked on any day in the last 2 months
- Past daily smoker vs. native ITS
- Total Nicotine Dependence Syndrome Scale (NDSS)⁷⁹ Score
- Wisconsin Inventory of Smoking Dependence Motives (WISDM)⁸⁰ Primary Dependence Motives Scale + Tolerance Item
- exhaled breath carbon monoxide concentration from screening visit (ppm)
- Days per week smoked during 2 weeks pre-enrollment (from TLFB)
- CPD on days smoked during 2 weeks pre-enrollment (from TLFB)

2.3. Statistical analysis

The original analysis plan called for weighting to rebalance intentional over-sampling of African American smokers. However, this approach has not been implemented in similar studies, and detailed information about ITS prevalence by racial category in Western Pennsylvania is not available to determine appropriate weights. Randomization was stratified by menthol preference to ensure treatment group balance of study cigarettes used (and as a proxy to balance race), but stratification was not planned as part of the analysis.

2.3.1. Primary analysis

For regulatory purposes, a straightforward primary endpoint and approach were chosen to be comparable to earlier studies. The primary analysis examined treatment group differences in the change score (CPD weeks 9-10 post-randomization subtracted from CPD 2 weeks prior to randomization, when own-brand cigarettes were provided gratis), controlling for baseline CPD (same measure as used in change score) in a linear regression model. As described above (Methods section 2.2), the primary analysis was performed on datasets with multiple imputation of CPD data missing due to dropout. Parameter estimates and inference were combined over the 25 imputations using PROC MIANALYZE.⁸⁴ Descriptive statistics tables and graphical display summaries were used to ensure that model assumptions were satisfied, and that multiple imputation yielded reasonable values.

Additional analyses control for covariates (gender, race, daily smoker history) and assessed their potential role as treatment effect modifiers, based on hypothesized effects in the literature.^{47,87,88}

The single primary analysis is well-specified, and the subgroup analyses are secondary and limited (3 potential effect modifiers). Therefore, no adjustments for multiple comparisons were planned.

2.3.2. Secondary analyses

2.3.2.1 Longitudinal CPD trajectories

A secondary analysis examined CPD over 2-week periods throughout the study, using linear mixed effects models with random intercept and linear slope, to describe linear and quadratic trends in CPD. Statistical significance of the quadratic term can be used to clarify whether decreases in smoking are approaching extinction or levelling out.

In the longitudinal analysis time was a continuous variable, using the midpoint of 2-week periods (days -7, 7, 21, 35, 49, 63 relative to day of randomization). Random intercept and linear slope were included in each model (though slope did not show much variation not explained by fixed effects). Fixed effects were time, quadratic time (time*time), and interactions of treatment effect with both time variables. Wald p-values were examined for each interaction term to assess statistical significance, and separately for linear combinations of effects to address statistical significance of treatment*time effects separately for subgroups. When quadratic effects and interactions were not statistically significant, models were refit with only linear time and time by treatment interactions. Standard graphical displays of residuals and subject-level and observation-level influence statistics were examined to identify gross violations of regression assumptions (there were none).

2.3.2.2 Abstinence

Smoking cessation (abstinence during the 2-week endpoint assessment period) was identified as a secondary endpoint. Because the abstinence endpoint was meant to complement the primary endpoint, a different approach was used to maximize use of study data – counting backward 2 weeks from end of study rather than forward from

randomization. “Abstinence in weeks 9-10” was assessed from the observed CPD measure and reports of smoking on the day of the final visit, and was imputed as “non-abstinent” for dropouts, consistent with standards for assessing abstinence in clinical trials.^{89,90} Subjects were also considered non-abstinent if urinary cotinine exceeded 100 ng/mL or exhaled CO exceeded 5 ppm, which would indicate smoking. Treatment group differences in abstinence were modeled using a logistic regression model, controlling for baseline CPD.

2.3.2.3 Sensitivity analysis: non-research cigarettes and other nicotine-containing products, and differences in urinary cotinine

Assuming that a treatment group difference was found in the ITT analysis with CPD as endpoint, a first set of sensitivity analyses focused on use of non-research cigarettes and other nicotine-containing products. Separate sensitivity analyses conducted using observed data (as another check of robustness of treatment effects to study assumptions):

1. excluded *non-research cigarettes* (which would not be legally available if the FDA implemented a VLNCC policy)
2. excluded *subjects* who reported $\geq 10\%$ use of non-research cigarettes (per Veldheer et al.);⁹¹ this group accounted for 69% of all conventional, non-research cigarettes reported in the study.
3. excluded *subjects* who increased use of other sources of nicotine post-randomization on average by more than one unit/2 weeks post-randomization. (Note that frequent use of e-cigarettes or other sources of nicotine was an exclusion criterion for the study.)

Non-research (conventional) cigarettes were reported separately, but in the same manner, as research cigarettes for each consumption measure (phone IVR, TLFB recall questionnaire, and butt collection). Use of e-cigarettes, nicotine replacement products, smokeless tobacco, pipes, cigars and hookah was reported in IVR and TLFB. Any usage of any other source of nicotine (OSN) was counted as a unit, with partial units for partial e-cigarette cartridges. The higher number of units from IVR and TLFB was used to summarize daily OSN.

Again assuming a treatment group difference would be found in the ITT analysis with CPD as endpoint, a second sensitivity analysis was planned to examine treatment group differences in urinary cotinine, which does not depend on self-report or collection of butts. Total cotinine concentration (ng/mL) was assessed from urine samples at the screening visit and 2, 6, and 10 weeks post-randomization. Samples were frozen and stored at -20°C , then batch-analyzed by liquid chromatography–mass spectrometry at the University of Minnesota laboratory of Dr. Stephen Hecht (same laboratory as for the prior RCT of VLNCC in daily smokers).¹ One analysis used cotinine measures to identify “cheaters” who were deemed likely to have smoked conventional cigarettes (Supplement 2.4) and repeated the primary analysis (with difference score as endpoint) using observed CPD data and excluding the cotinine-identified “cheaters”.

2.3.3 Additional analyses

2.3.3.1 Factors predicting post-randomization loss to observation (dropout)

Logistic regression and proportional hazards regression were used to identify subject features related to loss to observation after randomization. This exploratory analysis was intended to complement the multiple imputation of CPD outcome data, to clarify whether potential effect modifiers (gender, race, prior smoking history) were subject to different rates of loss to observation, and to inform planning for future studies. Each logistic regression model predicting loss to observation controlled for baseline CPD and treatment group, then evaluated gender, race, and past daily smoking (yes/no). Proportional hazards regression (endpoint: time to loss to observation) was examined with baseline CPD as a covariate and ongoing CPD as a time-varying covariate. In other words, this model examined whether change in CPD (possibly as a result of treatment) was related to subsequent loss to the study.

2.3.3.2 Exit interview data

At exit, participants were asked to guess which cigarettes they had been randomly assigned. Accuracy in guessing correctly was compared by treatment group using a Chi-square test.

Treatment group differences in the end-of-study intention to quit rating were assessed by t-tests using observed data.

2.4. Algorithm for identifying 'cheating' (smoking of conventional cigarettes) in ITS switched to VLNCCs

Background: An algorithm for identifying cheating by daily smokers switched to VLNCCs

Prior studies of VLNCCs among daily smokers of 10 or more cigarettes per day⁹² have suggested that a substantial fraction of such smokers "cheated" during the study by smoking regular commercial cigarettes in lieu of, or supplementing the assigned VLNCCs. Benowitz and colleagues⁹³ have proposed an algorithm for using observed values of cotinine, nicotine's major metabolite, to identify participants engaged in such cheating, by identifying those with cotinine values that are implausible without cheating. The algorithm is conceptually simple. It suggests computing the cotinine-per-cigarette ratio (literally $\frac{\text{cotinine-concentration}}{\text{cigarettes-per-day}}$) at baseline, when smoking normal cigarettes, and using this as a basis for computing the expected cotinine levels under VLNCCs. When the observed levels are substantially in excess of the expected cotinine levels, cheating is assumed to have occurred. (Specifically, the cotinine-per-cigarettes ratio is reduced in proportion to the nicotine reduction in the VLNCCs, a 4x allowance is made to account for any compensation that may occur, and values exceeding this threshold are presumed to be the result of cheating.)

Applying cheating the algorithm to ITS

Conceptually, this approach should also apply to ITS. However, there are significant complications that attend its application to ITS.

The effect of smoking recency. The idea of a cotinine/cigarettes-per-day ratio as an indicator of an individual's nicotine intake makes sense when both cigarettes-per-day (CPD) and cotinine values are at steady state, and thus can be regarded as stable quantities that characterize the individual. Among ITS, neither of these quantities is at steady state. Among ITS, CPD varies widely from day to day, from zero (by definition: some days are non-smoking days) to 20 or higher. The mean CPD doesn't really capture this dynamic. Because of this variation, cotinine values will also fluctuate. Cotinine has an average half-life of about 16-20 hours.⁹⁴ Thus, depending on how recently the person smoked, as well as how much, cotinine levels will vary from non-smoker levels to levels seen in heavy smokers. Consider an ITS who smoked 15 cigarettes the day before the urine sample was taken, and not at all the previous two days (a pattern of 0, 0, 15, average CPD=5): They will likely have fairly high cotinine levels, in the daily-smoker range, because of the recent heavy smoking. Consider the same smoker, but sampled two days later, after two days of abstinence (pattern = 15, 0, 0, same average CPD=5): their cotinine level will be quite low, perhaps approximating non-smoker levels. So, any calculus relating cotinine to cigarette consumption has to consider recency of smoking.

We have published an approach to addressing this by including recency information in the calculation of a weighted CPD (wCPD) when evaluating its relationship to cotinine.⁹⁵ Specifically, the approach weights the more recent days' smoking more heavily, halving the contribution for each preceding day. (The weights for the prior 3 days were set at 2, 1, 0.5, roughly approximating the half-life as a day. The weighted average is then taken by dividing the weighted sum by 3.5, the sum of the weights.) On this calculus, the pattern 0, 0, 15 yields a wCPD of 8.6, while the pattern 15, 0, 0 yields a wCPD of 2.0, reflecting the difference in how recently the 15-cigarette day occurred. (Incidentally, if the 15 cigarettes were distributed evenly, as 5, 5, 5, this would yield the expected intermediate wCPD of 5.)

The relationship between cigarette consumption and cotinine. The cotinine/cigarette algorithm proposed by Benowitz has the virtue of simplicity. But it actually makes a very strong assumption about the relationship of cotinine to cigarette consumption: that it is described by a straight 45-degree line with an intercept of zero. Empirical data clearly demonstrate that this assumption is untrue. Both in daily smokers^{95,96} and among ITS,⁹⁵ the relationship is non-linear and does not have an intercept of zero. It seems most appropriate to use an empirically-derived equation to model the cotinine-per-cigarette relationship, rather than an idealized but known-not-true equation.

In the present ITS baseline data, we have found that, while the relationship of wCPD to cotinine is non-linear, it can be made linear by using the logged values. Indeed, the correlation of $\log(\text{cotinine})$ to $\log(\text{wCPD})$ is linear and strong, with a correlation of 0.74 ($R^2=0.55$). This suggests that this equation, based on baseline smoking of commercial own-brand cigarettes, would be useful in modeling expected cotinine values in VLNCC treatment. Following the logic of Benowitz, the model-predicted values are discounted by 90% (the percent reduction in nicotine delivery from the VLNCCs) during treatment with VLNCCs, and a 4x allowance is made for compensation. If the observed cotinine values exceed that limit, the person is suspected to be cheating.

Ethnic variation in cotinine. The approach thus far assumes that the relationship between cigarette consumption and cotinine is the same for all groups. However, it is known to vary by ethnicity. In particular, it is known to be different among African-American smokers than among Caucasians: African-Americans have higher cotinine values

at lower levels of smoking.^{43,97} (There are also variations within ethnic groups, some accounted for by a genetic variation in the enzyme CYP2A6, which controls nicotine metabolism,⁹⁸ but that is beyond the present scope, as this genetic variation was not assessed in this study.) Failing to account for this may bias the algorithm towards more often labeling African-Americans as cheaters.

Indeed, in these ITS data, the relationship between wCPD and cotinine (in the logs) is significantly different for African-Americans vs other ethnicities ($p = 0.005$); the relationship is 'flatter' ($\beta = 0.93$ for AA vs $\beta = 1.71$ for all others) and less predictive for African-Americans. (Caucasians and other ethnicities yield nearly identical estimates so can be accommodated by a single equation.) The solution is to use two different equations, with African-Americans and others each being modeled by their group-specific equations.

Individual variations vs. cheating. Prediction of cotinine from wCPD is good, but not perfect. Thus, even at baseline, when subjects are smoking commercial cigarettes, and when the prediction equation is being applied to the very data used to generate the equation, some individuals' observed cotinine values will fall beyond the limits defined by the algorithm described above. This may be due to unmeasured individual variations in smoking intensity, nicotine and cotinine metabolism, dilution of the urine, or error in specifying wCPD (e.g., if the person smoked more than they reported). If an individual already falls outside the predicted limit at baseline, their falling outside the limit again later, after randomization, is uninformative. Accordingly, such cases will not be considered indicators of cheating.

Environmental tobacco smoke exposure as a confound. An additional problem arises because, when smoking at very low levels (and/or not recently), the cotinine levels of ITS can fall in the range of cotinine values seen in non-smokers who are exposed to environmental tobacco smoke (ETS). Foulds et al.⁹⁹ have noted this problem, even among daily smokers, and have argued the need to incorporate potential ETS exposure into algorithms for detecting cheating. According to the Society for Research on Nicotine and Tobacco Subcommittee on Biochemical Verification,¹⁰⁰ levels of urinary free cotinine above 50 ng/ml (equivalent to total cotinine levels of 160)¹⁰¹ are consistent with active smoking, with levels below that consistent ETS exposure. Accordingly, observed total cotinine levels below 160 are not considered indications of cheating, even if they meet other algorithm criteria.

Summary

- Regression equations will be developed, based on baseline data, relating recency-weighted CPD with total urinary cotinine (in the logs). Separate equations will be developed for African-American participants and those of other ethnicities.
- Participants will be considered to have exceeded the expected limit when their observed cotinine exceeds 4x their predicted cotinine. Predicted cotinine for participants switched to VLNCC cigarettes will be discounted by 90%, to account for the 90% nominal reduction in nicotine delivery from VLNCCs.
- Exceeding the expected limit will be considered to indicate cheating during treatment, unless the actual level is below 160 ng/ml (the ETS threshold), or the participant's cotinine exceeded the expected limit even at baseline.
- We can report and compare the percentage of participants in the VLNCC and NNCC groups that were identified as cheating (ever, and by number of occasions/visits). As we did with self-reported cheating, we can repeat the main analysis with and without cheaters. Since cheating can vary over time, we can re-analyze the data cutting out the 2-week blocks in which cheating was indicated, while retaining other data from the offending participant.

¹ In comparing cotinine values observed in this study, it is important to keep in mind that these are total cotinine levels assayed in urine. Most publications report free cotinine rather than total cotinine (which includes glucuronidated cotinine). Murphy et al.¹⁰¹ showed that total cotinine averaged 3.22 x free cotinine; this ratio is used as a multiplier when converting free cotinine values to total cotinine. Urinary cotinine concentrations are higher than serum or salivary, so the values are not interchangeable. Where literature referencing salivary or serum concentrations are referenced, an appropriate conversion factor is used.

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