University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
TEMPLATE WITH GUIDANCE

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Study Title: Impact of e-cigarette nicotine concentration on compensation, cigarette smoking, and biomarkers of exposure and harm in diverse smokers

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I. Purpose, Background and Rationale

A. Aim and Hypotheses

1. E-cigarettes (ECs) are projected to exceed combustible cigarette use within two years. Policy makers, health officials, and regulators are concerned that newer nicotine salt-based Ecs that use high concentrations of nicotine in their e-liquids are a major reason for this rapid growth in use. The US Food and Drug Administration (FDA) has regulatory authority to set appropriate tobacco product standards to protect public health and has shown interest in exploring a product standard limiting the level of nicotine in e-liquids. While this regulatory consideration has merit, emerging research suggests it may be misguided, leading to a product that is just as addictive but more harmful. Specifically, among users of earlier, freebase nicotine Ecs (i.e., cig-a-like, tank systems), use of low nicotine e-liquids was associated with a 9-fold increase in e-liquid consumption and all of its related toxicants, likely due to compensatory puffing. The consequences of consuming more e-liquid because of lower nicotine concentration remains an important knowledge gap. Moreover, the National Academies of Science, Engineering, and Medicine have concluded that completely substituting Ecs for cigarettes results in less short-term harm than continued smoking, but the impact of low versus high nicotine concentration e-liquids on a smokers' ability to completely switch to Ecs (versus become 'dual users' or continue smoking) is currently unknown. African American (AA) smokers, who take larger puffs, inhale more intensely, and extract more nicotine and harmful constituents per cigarette smoked, may be particularly impacted by nicotine product standards placed on EC – i.e., greater compensatory puffing and more e-liquid and related toxicant consumption at lower e-liquid concentrations. Unfortunately, the vast majority of information on Ecs and potential product standards come from white populations and have largely ignored African American (AA) smokers who bear a disproportionate burden of tobacco-related morbidity and mortality. As the FDA considers regulatory action to limit the level of nicotine in e-liquids to protect public health, it is critical that research considers vulnerable populations and does not widen disparities.

   Our long-term goal is to inform a tobacco landscape that will minimize tobacco-related harms and downstream health inequities. The overall objective of this application is to understand the impact of e-liquid nicotine concentration on compensatory puffing, EC and cigarette use patterns (exclusive EC, dual EC-cig, exclusive cig), and resultant exposure to biomarkers of harm among AA and white smokers. Adult AA and white

smokers will complete two study phases. In Phase 1, using a randomized crossover design, participants will complete two standardized, 10-puff vaping bouts over 5 mins followed by a 60-minute ad libitum vaping session, using two e-liquids that differ only by nicotine concentration (5% vs. 1.8%) to examine the effect of nicotine concentration on in-lab compensatory puffing, nicotine exposure, and e-liquid consumption. In Phase 2, the same participants will be randomized to 5% or 1.8% nicotine e-liquid and instructed to switch completely for 6 weeks to examine the impact of nicotine concentration on short-term, real-world EC use patterns and related biomarkers of exposure (e.g., exhaled carbon monoxide, NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol), lung inflammatory markers). Our central hypothesis is that, compared to the high nicotine concentration, while vaping the low nicotine concentration, users will engage in compensatory puffing, resulting in greater e-liquid consumption (Phase 1). Moreover, rates of dual use and continued smoking will be higher for the low (versus high) nicotine concentration and will result in greater exposure to toxicants (Phase 2).

2. Aim 1 (Human laboratory): To examine the effect of nicotine concentration on compensation measured via nicotine exposure, puffing behavior, and e-liquid consumption. H1a: Compared to the 5% nicotine concentration, while vaping the 1.8% concentration, smokers will evidence greater total inhaled volume, and (H1b) greater consumption of e-liquid (by pod weight) (H1c) but achieve similar levels of nicotine exposure.

   Aim 2 (Randomized trial): To assess the comparative efficacy of 1.8% vs. 5% nicotine concentration on real-world EC use pattern (exclusive EC, dual EC-cig, exclusive cig). H2a: After a 6-week trial of EC, rates of complete substitution (i.e., percent exclusive EC) will be significantly greater for participants randomized to 5% (compared to 1.8%) nicotine e-liquid. H2b: Rates of dual EC-cig and exclusive cigarette smoking will be greater for participants randomized to 1.8% (compared to 5%) e-liquid.

   Aim 3 (Randomized trial): To understand the comparative exposure to non-nicotine constituents as a function of nicotine concentration. H3a: The degree of changes in exposure will be directly associated with the degree of substitution with EC—greater levels of substitution will confer a larger decrease in exposure. H2b: Among those who completely substitute Ecs, 5% users (vs. 1.8%) will have lower toxicant levels.

B. Background and Significance

1. Study Significance and literature review:

   Our study will be the first to assess the impact of nicotine concentration on compensatory puffing (total inhaled volume), nicotine delivery, and switch patterns (percent exclusive EC, dual cig-EC, and cig only users) with an explicit focus on AA and White smokers. It will provide the first available evidence on potential differential change in these factors for AA and Whites and offer rich data to the FDA that will inform evidence-based regulation of a nicotine standard that maximizes public health benefit and limits potential harm among diverse smokers.

   Rise of e-cigarettes (Ecs) and the impact on public health: Millions of white US adults and youth now use Ecs[27,28] that deliver fewer carcinogens, volatile organic

compounds, and other harmful toxicants than traditional cigarettes.[29,30] EC data show lower levels of tobacco-related toxicants[29,31-34] and led the National Academies of Science, Engineering, and Medicine to conclude in their groundbreaking report that, if used exclusively, Ecs are likely to pose less risk to an individual than cigarettes and, to the extent that adult smokers completely switch to Ecs, cause less short-term harm than cigarettes.[35] NSBEs have again disrupted the tobacco landscape. NSBE e-liquids are available in high nicotine concentrations (up to 5%),[36] higher than most other EC e-liquids on the market (0.3%-2.4%).[37-39] These products use protonated nicotine[36], which make the high nicotine e-liquids palatable and reduce irritation,[40] likely facilitating user initiation.

Tobacco and EC use among African Americans (AA): AA smoke fewer days per month, fewer cigarettes per day (CPD)[41] (1 to 10), and are less likely to be heavy smokers than whites.[41] Despite smoking fewer cigarettes per day (CPD)[41] which should result in reduced negative health consequences, AA smokers are exposed to greater levels of tobacco-related toxicants,[42] have higher cardiovascular and cancer-disease risk at lower smoking levels and bear a disproportionate burden of smoking-related diseases.[43,44] AA smokers take larger puffs,[45] inhale more intensely,[46] and extract 30% more nicotine and harmful constituents per cigarette compared to whites.[47] With the emergence of Ecs, important questions regarding the impact on AA smokers arise. Specifically, as more AA smokers switch to Ecs, it is not known how they will use the products. One possibility is that AA smokers may engage in fewer vaping sessions but intake more e-liquid (through increased topography), which is not harmless, compared to whites to reach comparable nicotine exposure. However, almost all the information on Ecs and potential product standards come from white populations. As the FDA considers regulatory action to limit the level of nicotine in e-liquids to protect public health, it is imperative that research be inclusive of populations who bear a disproportionate burden of tobacco-related morbidity and mortality. Given the dearth of preliminary data, subanalyses of race will be exploratory in nature.

Nicotine and other EC constituents: Nicotine is the addictive component in tobacco products,[48] including Ecs. While nicotine contributes to dependence and maintains tobacco product use,[49] health concerns regarding nicotine primarily arise due to the type of delivery device and route of administration. In Ecs, nicotine is delivered in a solution of propylene glycol (PG), vegetable glycerin (VG), and flavorants.[50] E-liquid solutions may also deliver other non-nicotine constituents and harmful and potentially harmful constituents including heavy metals, volatile organic compounds (VOCs), and low levels of polycyclic aromatic hydrocarbons (PAHs).[51]

Nicotine concentration and compensation in AA and white users: Policy makers, researchers, and the public have expressed concern regarding the high levels of nicotine in NSBEs and have suggested product standards that would place a ceiling on allowable nicotine concentrations to reduce public health harms[52], similar to the European Union Tobacco Products Directive that restricts nicotine strength to ≤ 20mg/mL[53] (i.e., 2%) and recent state-level recommendations to implement a similar rule.[54-57] While such restrictions may have some merit, concerns regarding high nicotine concentrations and initially proposed product standards may be misguided and have never been tested. In a

study of white tank and mod system EC users, use of low nicotine devices resulted in a 9-fold increase in e-liquid consumption despite equivalent nicotine exposure,[58] a finding that has been replicated in the literature.[59-61] These data suggest that white users of older, low nicotine devices likely engage in compensatory puffing to achieve the same level of nicotine as high nicotine EC users,[58-61] although objective measures of puff topography were not collected. Laboratory emissions testing research has shown that at low (vs. high) nicotine concentrations, a greater amount of e-liquid must be aerosolized to achieve cigarette-like levels of nicotine yield. Importantly, greater e-liquid aerosolization generated by higher temperature devices resulted in emissions of higher levels of carbonyl compounds, including known respiratory irritants and carcinogens in humans.[62] If replicated in humans, EC users would likely be exposed to higher levels of PG, VG, flavorants, and other constituents. Given their differential puffing patterns (greater number of puffs[45] and more intense puffing[46]), AA smokers may engage in even greater e-liquid consumption and, as a result, be exposed to greater levels of non-nicotine constituents compared to whites. If users engage in compensatory puffing when using low levels of nicotine, they are being exposed to more e-liquid, and placing a ceiling on allowable levels of nicotine may inadvertently increase exposure to non-nicotine constituents, particularly among AA smokers. Unlike very low nicotine concentration (VLNC) cigarettes,[63-65] users of low nicotine Ecs have not been shown to reduce their use in response to the reduced levels of nicotine.[58-61] Human health effects of exposure to larger volumes of EC aerosols remain unknown and of concern.

The role of nicotine concentration in EC use trajectory in smokers: Ecs are a potential harm reduction tool, particularly if used exclusively by smokers who are unable or unwilling to quit smoking combustible cigarettes. Ecs pose less short-term risk than cigarettes and, particularly when used exclusively, cause less harm than cigarettes.[35] Therefore, product standards should facilitate a transition from combustible smoking to exclusive EC use among adults. Only a handful of randomized trials have examined quit rates using Ecs and none have directly compared the impact of EC nicotine strengths on use trajectory. Existing studies indicate that when smokers were provided a cig-a-like with 7.5mg (~.75%) nicotine e-liquid, smokers achieved quit rates of only 11% at 12 weeks.[66] However, 16mg (~1.6%)[67] and 18mg (~1.8%)[68] e-liquids produced quit rates of 21.5% and 35%, respectively. Dr. Nollen's (primary mentor) switching trial of AA and Latinx smokers is the only trial to examine rates of abstinence from Ecs using a NSBE and showed that 28% of smokers were exclusive EC users at week 6.[69] Together, these data suggest that rates of cigarette abstinence increase with increases in nicotine concentration. However, a head-to-head comparison of the impact of EC nicotine concentration on EC and cigarette use is a critical next step to inform nicotine product standards in Ecs.

Relevance to tobacco regulatory science: The FDA deemed that Ecs are under their regulatory authority, thus allowing FDA to implement policy decisions regarding EC product standards, including nicotine.[70] The proposed research will provide urgently needed data regarding nicotine product standards in Ecs and the impact on compensation, EC and cigarette use patterns, and resultant toxicant exposure among AA and white smokers. Indeed, FDA has noted that evidence-based nicotine product standards are a top priority, and that data are needed to inform the implementation of

policy specific to nicotine product standards in Ecs. There remains an urgent need to understand how racially diverse smokers respond to and are impacted by potential product standards.

2. Dr. Leavens' recently completed randomized crossover, human laboratory pilot study (N = 10) of nicotine exposure of three Ecs (tank system, mod style, NSBE) and cigarettes showed that use of NSBEs led to shorter time to maximum plasma nicotine concentration than cigarettes. While the study provides initial evidence regarding nicotine delivery of NSBEs, participants were only provided 5% nicotine Ecs and markers of harm were not measured.

C. Rationale

Dr. Leavens' recently completed randomized crossover, human laboratory pilot study (N = 10) of nicotine exposure of three Ecs (tank system, mod style, NSBE) and cigarettes showed that use of NSBEs led to shorter time to maximum plasma nicotine concentration than cigarettes. While the study provides initial evidence regarding nicotine delivery of NSBEs, participants were only provided 5% nicotine Ecs and markers of harm were not measured. Dr. Nollen has recently completed the first EC switching study with AA adult daily smokers.[69] Major findings include high interest in switching to EC among AA smokers, high utilization of NSBE, and significant reductions in cancer, respiratory, and cardiovascular markers among the EC group. Participants all received NSBE containing 5% nicotine. The health effect of 1.8% versus 5% nicotine remains a critical gap that will be addressed through the proposed research plan. Dr. Wagener completed one of the first studies to show that use of low-nicotine Ecs was associated with greater e-liquid consumption compared to high-nicotine Ecs.[58] However, this study included only whites, did not include topography, and was conducted with devices that are now considered outdated. NSBE contain product features that more closely mimic the experience of smoking a cigarette (e.g., nicotine boost)[6,71,72]; these features may impact the rate of compensation and, subsequently, the amount of e-liquid consumed, and are gaps that will be addressed in the proposed research. The research will merge Dr. Leavens' foundational knowledge of human laboratory studies of novel products with Dr. Nollen's expertise in tobacco-related disparities among AA using randomized trial methods. Additionally, Drs. Wagener and Benowitz will supplement these core components by providing expertise and training in tobacco regulatory science and biomarkers of harm of EC use. All mentors will provide training in grantsmanship and professional development.

II. Research Plan and Design

A. Study Objectives: This study will investigate the impact of EC e-liquid nicotine concentration (1.8% vs. 5%) on compensatory puffing and e-liquid consumption, use patterns, and resultant changes in biomarkers of exposure, among AA and white smokers using complementary human laboratory and randomized trial methods.

B. Study Type and Design:

Adult AA (n = 24) and white (n = 24) smokers (stratified on CPD) will complete two study phases. Phase 1 (P1): 2-visit human laboratory trial with double-blind, randomized crossover design. Phase 2 (P2): 6-week, randomized substitution trial. Participants in P2 will be the same participants that completed P1. Both phases are necessary: Phase 1 will provide data on compensatory puffing and nicotine exposure, while Phase 2 will provide information on real-world use patterns and related short-term health effects. These two phases, combined,

provide a more comprehensive picture of the impact of nicotine product standards on population-level health than either phase alone.

C. Sample size, statistical methods, and power calculation

1. 48 smokers will complete phase 1, a randomized crossover trial. In order to have 48 "completers" of phase 1, we will enroll about 75 participants and stop enrollment once 48 participants have completed phase 1. Only 48 participants will complete phases 1 and 2. Participants will be randomized 1:1 to the order in which they use the two nicotine concentrations. For the randomized trial, they will be randomized 1:1 by computer generated random number to switch to 1.8% or 5% nicotine e-liquid for 6 weeks. Randomization will be determined by computer-generated random numbers. Randomization assignments will be placed in sealed envelopes with sequential study ID numbers. After baseline data collection during phase 1 has been completed, the research assistant will select the sequential study ID number to determine the randomization assignment. This procedure will be conducted again at the beginning of phase 2 (randomized trial).

2. Both phases of the trial will be double blind. Randomization will be determined by computer-generated random numbers. Randomization assignments will be placed in sealed envelopes with sequential study ID numbers. After baseline data collection during phase 1 has been completed, the research assistant will select the sequential study ID number to determine the randomization assignment. This procedure will be conducted again at the beginning of phase 2 (randomized trial).

3. The purpose of a K01 is to build a body of preliminary evidence and determine effect sizes for a fully powered R01 trial. With this in mind, the proposed K01 is fully powered to detect differences in change in puff volume between 1.8% and 5% nicotine concentration e-liquids for the sample overall (Aim 1). For this aim and all subsequent aims, we will conduct subset analyses by race. Phase 1: The primary outcome for P1 is total inhaled volume. We have determined these anticipated effect sizes based on pilot data from Dr. Leavens' study on puff topography among users of 5% NSBE e-liquid during a standard PK assessment and existing data regarding puff topography among users of moderate (3%) and low (1.8%) nicotine concentration e-liquid in earlier generation Ecs (Hiler et al., 2017).[59] We are estimating topography for the lower nicotine concentration based on a range of values. Given that we do not have estimates for the NSBE, we are using the smaller, clinically meaningful difference. These estimates suggest an anticipated mean difference in total inhaled volume between the 1.8% and 5.0% nicotine concentrations of 70 mL (12% increase) and 186 mL (27% increase). We are basing our sample on the smaller, clinically meaningful difference (12% increase) which results in an effect size of 0.74. Based on this information, 48 participants we will achieve >90% power to detect an increase of 70mL in total inhaled volume during the PK assessment using two-sided tests with 0.05 alpha level, assuming a mean total volume of 679mL and 749mL for the high and low nicotine e-liquid, respectively, and a common standard deviation of 200 mL. We will subsequently conduct separate analyses by race (AA = 24; White = 24). Based upon the previous assumptions, this results in the ability to detect an effect size of 1.08 or larger within each subset. We will conduct separate analyses by race because, while we anticipate AA will have globally greater puff

volume[90], there are no data that suggest a differential change in inhaled volume between AA and white smokers. Based on previous research, there is potential for a main effect of race; however, a study of AA and white smokers stratified on baseline cigarettes per day has not been conducted. This study will be the first head-to-head, controlled, prospectively designed study to assess if there is the potential for a main effect of race. This sample estimate is based on our most variable outcome; therefore, we anticipate being overpowered to detect effects in the overall sample related to nicotine exposure (secondary outcome) which will show much less variability. Subsequently, after a standard 1-week washout period, subjects will return to be randomized to 1.8% or 5% nicotine e-liquid and instructed to completely substitute the EC for cigarettes. Based upon prior retention, we expect to randomize at least 40 subjects in P2. Further, based on Dr. Nollen's past trials,[25,91-93] we do not anticipate differential attrition by race. Prior studies conducted by the research team have retention of >90% in multi-year trials examining AA and white smokers. Phase 2: The primary outcome for P2 is the proportion of participants that achieve biochemically-verified complete substitution (i.e., exclusive EC use) at 6-weeks for 1.8% and 5% nicotine e-liquid. Based on Dr. Nollen's pilot study examining complete substitution among AA smokers using 5% nicotine salt e-liquid[69] and similar studies of Whites using 5% nicotine salt e-liquid in the literature, 94 we anticipate that ~30% of the sample overall will completely switch at 6-week follow-up in the 5% nicotine group and fewer in the 1.8% nicotine group. Twenty participants in each nicotine concentration group will allow us to estimate the rate of complete substitution (exclusive EC use) with a margin of error < 0.20 for each nicotine concertation group to determine an effect size for a larger scale study in the future. The exploratory subset analyses within each race will provide estimates that will determine whether a definitive trial comparing races is warranted. We chose the largest sample size that we could accommodate with the time and cost constraints. Thus, we will initially recruit 48 subjects for P1 and expect at least 40 to return and be randomized for P2.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

Inclusion and exclusion criteria are designed to enroll relatively healthy adult smokers who predominately smoke cigarettes, have little history of EC experimentation, and are uninterested in quitting smoking in the next 6 months.

1. Inclusion criteria:
   1) identify as non-Hispanic white or non-Hispanic African American/Black
   2) willing to switch from smoking to e-cigarettes for 6 weeks
   3) speak and understand English
   4) smoke greater than or equal to 25 of the last 30 days for the past 3 months
   5) not previously used an e-cigarette for longer than 30 days
   6) exhaled carbon monoxide of greater than or equal to 6ppm at screener visit
   7) willing to abstain from marijuana for 12 hours prior to in-person lab visits
   8) willing to abstain from smoking and vaping for 12 hours prior to 3 in-person lab visits
2. Exclusion criteria:
   1) weekly use of an EC over the last six months
   2) use of tobacco products other than cigarettes on greater than or equal to 10 days in the past 30 days
   3) use of EC on more than 5 of the past 30 days
   4) current use of cessation medications

5) pregnant, planning to become pregnant, or breastfeeding
6) past 30 day hospitalization/ER visit for psychiatric issue, seizure, stroke, or new heart problem
7) recent history of cardiovascular or pulmonary events in the past three months
8) treatment for alcohol or drug dependence in the past year
9) household member currently or previously enrolled in the study
10) current enrollment in a program aimed at changing smoking patterns

1. Withdrawal/Termination criteria:
   Participants will be instructed to present to the lab nicotine deprived (12 hours abstinent) for the first study visit. If participants present to the first study visit with an eCO greater than 12 ppm or they report other nicotine use two times, they will be withdrawn from the study. Participants will complete a paper or online log of their practice. If they do not practice with each nicotine concentration prior to visit one, they will be sent home and again instructed to practice with each pod. After two failed practice periods, participants will be removed from the study.

2. Participants will not be allowed to participate in another research study that aims to alter their tobacco use during this research study.

E. **Specific methods and techniques used throughout the study**

1. Laboratory tests:

   At two human laboratory visits, blood will be collected for nicotine analysis via a blood draw from the participant's arm. Participants will have an intravenous catheter placed in their arm for collection of blood (plasma nicotine). Blood sampling for changes in nicotine levels will occur at -5 (baseline), 5 (post 10-puff bout), 7, 15 (beginning of ad lib session), 45, and 75 (post-session) minutes. No more than 7 mL of blood will be drawn per draw. Blood will be processed and analyzed for nicotine and cotinine levels. If consent is provided for banking, samples will be banked for possible future analysis.

   Human Laboratory Assessments (see Table 1 for timing):

Topography. Puff topography will be used to objectively measure EC puffing patterns. Puff topography is a sensitive measure of substance self-administration.[81,83] Puff topography will be measured throughout the EC session via a pressure flow sensor in the EC mouthpiece, whereby inhalation-induced pressure changes are amplified, digitized, and sampled. Software converts signals to airflow (ml/s) and produces measures of puff volume, total puff volume, puff number, and inter-puff-interval. The topography device to be used in this study has been successfully used by Drs. Leavens and Wagener.[1,4] During both phases of the study, the study e-cigarette will be equipped with an integrated puff topography device that allows for collection of topography data in the lab and in the participant's natural environment. Participants will be provided the device and a phone with highly limited capabilities. Specifically, participants will only be able to change the brightness, connect to WIFI, and access the app for data transfer. Otherwise, the phone is fully locked down. Participants will be asked to open the topography app daily and "sync" to transfer daily topography data. The data will be utilized in an exploratory fashion. Only topography data and no identifiable data are transferred via the mobile application.

Nicotine exposure. At both initial lab-based visits, blood samples will be collected at -5 (baseline), 5 (post 10-puff bout), 7, 15 (beginning of ad lib session), 45, and 75 (post-session) minutes. Seven mL of blood will be collected during each blood draw. Samples will be analyzed according to standardized methods[84] by Dr. Na Zhang at KUMC. Blood will be analyzed for nicotine content to assess nicotine exposure during the session.

Biomarkers. eCO levels will be measured at final screening and both visits. At final screening, eCO ≥ 6ppm will be used to verify smoking status. At study visits, eCO ≤ 12 ppm will be used to verify tobacco abstinence.

Other self-report assessments. The EC-adapted Tiffany-Drobes Questionnaire on Smoking Urges (QSU-brief)[85] and Minnesota Nicotine Withdrawal Scale (MNWS)[65] will measure smoking urges and nicotine withdrawal, respectively, during each blood draw. The modified Product Evaluation Scale[86] (mPES) will assess satisfaction, subjective effects, and sensory experiences (e.g., taste) at the conclusion of each ad lib session.

Table 1. Phase 2 assessments and timing	W0	W1	W2	W3	W4	W5	W6
Baseline assessments and tobacco use							
Demographics, medical, tobacco use, substance use history							
Nicotine dependence	X						X
Timeline Followback (TLFB) of EC/tobacco use	X	X	X	X	X	X	X
Puff topography	X	X	X	X	X	X	X
Assessments of nicotine exposure							
Plasma nicotine (acute nicotine exposure)							
Urinary cotinine (prolonged nicotine exposure)	X						X
Assessment of health outcomes & biomarkers							
eCO (acute smoke exposure)	X						X
NNAL (carcinogen exposure)	X						X
Spirometry (lung function)	X						X
Urine collection for later analysis of Acrolein & Acrylonitrile	X						X
Blood pressure (cardiovascular effects)	X						X
Respiratory symptoms checklist (respiratory effects)	X						X
E-liquid consumption (by pod weight)	X						X
Other self-report assessments							
Craving/Withdrawal (QSU-brief and MNWS)	X						X

Randomized Trial Assessments (see Table 1 for timing):

Nicotine exposure. Prolonged nicotine exposure will be measured using urinary cotinine at wks 0 & 6. Cotinine captures nicotine from all nicotine products and will allow us to understand whether participants maintain, reduce, or increase their nicotine exposure over the course of P2. Participants will bring their empty and partially used pods to each study visit to be weighed for measurement of e-liquid consumption.

Health outcomes and biomarkers. eCO levels will be measured at wks 0 & 6. eCO ≤ 6 ppm will be used to verify use trajectory at wks 0 & 6. NNAL, spirometry, blood pressure, and respiratory symptoms will be measured at wks 0 & 6 to assess health outcomes and biomarkers between users of 1.8% vs. 5% nicotine e-liquid. Specimen samples will be banked for later analysis of VOCs, PAHs, and other new and emerging biomarkers of interest, including acrolein and acrylonitrile.

EC use trajectory (Aim 2). The 7-day Timeline Followback Interview (TLFB)[87,88] will be used to assess the comparative effectiveness of nicotine concentration on participants EC use trajectory at the end of the 6-week trial of EC. The primary outcome of P2 is the proportion of smokers who are exclusive EC users at wk 6 for 1.8% and 5% nicotine concentrations. Exclusive EC users are defined as those who reported any use of ECs, no use of cigarettes in the past 7 days, and who have a CO < 6 ppm.[89] Dual users are defined as those who reported any use of ECs and any use of cigarettes in the past 7 days. Those who reported no use of cigarettes in the past 7 days but who have a CO > 6 at week 6 will be classified as dual users. Exclusive cigarette users are those who report no use of ECs and any use of cigarettes in the past 7 days. A fourth group, no use of ECs or combustible cigarettes, may occur.

Other self-report assessments. The QSU-brief and MNWS will be collected at wks 0 & 6.

2. Study Procedures:
    i. **Initial Screening:** The initial screen will review inclusion/exclusion criteria. Those eligible will be scheduled to complete final screening within 14 days.
    ii. **Final Screening and Enrollment:** Participants will be instructed to smoke as they normally would before the final screening. Final eligibility screening will be conducted in-person and will consist of completing a pregnancy test on women of childbearing age, exhaled carbon monoxide (eCO) measurement, and obtaining informed consent. Participants will complete baseline measures including demographics, medical, tobacco use, and substance use history and measurement of cotinine, NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol), eCO, spirometry, blood pressure, lung injury panel, and respiratory symptoms. E-liquid Flavor Sampling and Device Practice. At the final screening and enrollment visit, participants will be asked to sample two e-liquid flavors (Menthol and Tobacco; only commercially available flavors in pod-based NSBE) with 5% nicotine to determine flavor preference. Consistent with established methods, at the conclusion of the visit, participants will be provided with the Vuse EC device and two pods (one each of 1.8% and 5% nicotine) both in their preferred flavor to take home for 48 hours and instructed to practice using each pod 2-3 times per day for at least 15 minutes per episode between the screener visit and the first study visit. Participants will complete a paper or online log of their practice. If they do not practice with each nicotine concentration prior to visit one, they will be sent home and again instructed to practice with each pod. After two failed practice periods, participants will be removed from the study. We considered

other nicotine concentrations but chose 5% because it is the most commonly used NSBE concentration in market leaders like JUUL. We chose 1.8% because it reflects the UK nicotine standard and most likely potential regulatory decision.

b. **Phase 1 – Human Laboratory Procedures:** Participants will remain abstinent from nicotine/tobacco and alcohol for 12 hours prior to each study visit. Abstinence will be verified upon arriving at the laboratory by eCO (≤ 6 ppm). Pregnancy exclusion will be confirmed at each visit. Self-report will be used for EC abstinence, as there is currently no objective, reliable measure to verify non-use of these products within the time the participant will be present in the lab. Participants will have an intravenous catheter placed in their arm for collection of blood (plasma nicotine). They will be provided the Vuse Alto device with one of the two e-liquid concentrations (1.8% or 5%) and will complete the 10-puff standardized bout (pharmacokinetic assessment) followed by a 60-minute ad libitum vaping session. The combined 10-puff bout and ad-lib session are commonly used human laboratory methods for complete assessment of product selfadministration.[21,59,75-80] For the 10-puff bout, participants will be instructed to take one puff every 30 seconds for five minutes. Throughout the session, puff topography will be measured via a pressure sensor attached to the EC device[81], methods used by Dr. Leavens in past and ongoing studies.[1,4] During the vaping session, blood sampling will occur at -5 (pre-session), 5 (post 10-puff bout), 7, 15 (beginning of ad lib session), 45, and 75 (post-session) minutes. Following a minimum 48-hour standard washout period[1,4,59,78,82] during which they will smoke as they typically would, participants will repeat this procedure with the other nicotine concentration.

Phase 2 – Randomized Trial: Participants who complete P1 (~20 AA and 20 W based on retention in Dr. Nollen's trials) will undergo a 1-week washout period (week 3 in consent document) and be randomized (1:1) by computer-generated random numbers to 1.8% or 5% e-liquid. Randomization will be stratified within race on CPD. Substitution: Participants will be instructed to make a complete switch from cigarettes to ECs for 6 weeks. They will be provided with the Vuse EC and e-liquid and instructed to use it as they like but to refrain from use of other tobacco. Participants will complete in-person visits (wks 0 & 6) and will provide samples for biomarker assessment and complete self-report measures. Participants will bring all empty pods and those currently in use for weight measurement to quantify e-liquid consumption. Phone calls will occur between in-person visits (wks 1, 2, 3, 4, & 5) to confirm appointments, collect data on tobacco use, and support substitution.[69] Participants will be asked to use the topography-equipped e-cigarette and accompanying phone/mobile application throughout phase 2 (RCT). While participants will be instructed to completely switch to ECs during P2, research suggests some participants will have difficulty doing so. Therefore, we will collect information regarding use of other products.

3. Due to COVID-19 and the need to keep patients and researchers safe, we may use Zoom, a HIPAA compliant, university approved video conferencing software, during the visit. This will allow the participant to smoke freely (unmasked) while the researcher can watch and communicate with the participant via video conferencing from outside the clinic room. We will utilize Zoom as much as possible and only enter the room when necessary.

4. All procedures, tests, and visits are being performed solely for research purposes and are not billable to insurance.

5. Samples will be labeled only with a unique study identification number and only members of Dr. Leavens and Zhang's teams will have access to the samples. Samples will be disposed of one month after the final report is sent out to the Principal Investigator, unless participants agree to have their urine and/or blood stored for future testing.

6. **Timeline:** The proposed study is comfortably feasible in the K01 award period, allowing time for project start-up and dissemination (Table 1). Refinement of assessments, database creation, and IRB approval will take place in the first 6 months of Year 1. We expect to recruit 10 participants in the second 6 months of Year 1, 18 participants in Year 2, 14 in Year 3, and 6 in Year 4. No more than 5 participants (≤15 visits) will be recruited during each quarter of recruitment. This rate of accrual is commensurate with my past studies and other similar laboratory-based studies. Our final participant session will be in the second quarter of Year 4. Data analysis and manuscript preparation will take place in the 3rd and 4th quarters of Year 4 and throughout Year 5.

Study Timeline by Year and Quarter

	Year 1				Year 2				Year 3				Year 4				Year 5			
Periods (3 months)	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Preparing research staff and REDCap measures	x	x																		
Participant recruitment (*N* = 48)			5	5	4	5	5	4	4	4	3	3	3	3						
Phase 1 (PK assessment)			x	x	x	x	x	x	x	x	x	x	x	x						
Phase 2 (randomized trial)			x	x	x	x	x	x	x	x	x	x	x	x	x					
Processing/analysis of samples (P1)													x	x	x	x				
Processing/analysis of samples (P2)															x	x	x	x		
Data and manuscript preparation (P1)															x	x	x	x	x	x
Data and manuscript preparation (P2)															x	x	x	x	x	x

Note. P1 = Phase 1; P2 = Phase 2

F. Risk/benefit assessment:

1. Physical risk: The potential risks for this study are minimal. There is a slight risk of discomfort, bruising and infection with blood draw. Blood will be drawn by trained research staff. IVs will be placed and blood draws will be conducted by an RN or LPN. Sterile instruments will be used for blood draws, the participants skin will be cleaned with an alcohol wipe at the site of the needle stick.
2. Psychological risk: Risks for participants include those associated with the inconvenience of participation including answering surveys, providing blood samples, and completing multiple visits. To minimize the inconveniences associated with study participation we will review all data collection instruments and study procedures to minimize the number of items in our instruments and improve the accessibility and convenience of our study procedures. We anticipate using several methods to enhance convenience to participants, including providing rideshare services to and from all study visits and offering study visits throughout the day. Another risk is feeling pressured to be in the study, which we will track in order to monitor and will report as an adverse event. Finally, although very unlikely, some questions may make participants uncomfortable; participants are not required to answer questions they do not wish to.
3. Social risk: None
4. Economic risk: None
5. Potential benefit of participating in the study
    a. There are no direct benefits to participants for participating in this study
    b. If shown to be effective, our study could, in the future, minimize tobacco-related harms and downstream health inequities by exploring the effect of nicotine concentration on puffing patterns and biomarkers of harm. The data will inform FDA regulations that protect the public health, particularly that of smokers.
    c. The researchers hope that the information gathered from this research may be useful in informing regulatory decisions to benefit public health.

G. Location where study will be performed:

All Phase 1 visits will be run at the Clinical and Translational Science Unit-Swope (CTSU-Swope). The CTSU offers clinical space including two smoking rooms and is staffed by experienced RNs and medical assistants. Biospecimens including NNAL, urinary cotinine, plasma nicotine, and the lung injury panel will be processed and analyzed by Dr. Na Zhang and her team. Blood will be collected for plasma nicotine measurement during both study visits during Phase 1. All biospecimen samples are deidentified and stored in a -80 degree freezer. Names of participants will be kept separate from participant data. Only study research assistants and the PI will have the information that connects participant names and ID numbers. All electronic data will be numerically coded and stored in a password-protected database, on a password-protected computer in a secure research space. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication. No information will be stored locally on the laptop/tablet; all information will be stored securely in the secure data capture system. Research materials obtained from the participants include responses to questionnaires collected directly by our research team and entered directly within secure databases. Data will be stored in a password protected database and on password protected network storage. Consent forms will be stored in a separate locked filing cabinet or electronically on the secure database. Physiological measures include, urine, blood samples, and exhaled breath carbon monoxide. Research data will be obtained specifically for research purposes. Every effort will be made to maintain subject confidentiality, in accordance with HIPAA.

H. Collaboration (with another institution, if applicable): This research is being conducted as part of the PI's K01 award and is intended to provide training to establish independence. As such, the PI has mentors at the other institutions – The Ohio State University and UC San Francisco. No identifiable data will be shared with these mentors.

I. Single IRB Review for a Multi-site study (if applicable): N/A

J. Community-Based Participatory Research (if applicable): N/A

K. Personnel who will conduct the study, including:

Indicate, by title, who will be present during study procedure(s): Eleanor Leavens (PI), Nikki Nollen (co-I; mentor), Matthew Mayo (biostatistician), Tricia Snow (lead lab coordinator), Terri Tapp (research assistant), Leah Lambart (GRA), Stephanie Hiebert (GRA), Leo Byer (RA) , Dan Li (regulatory staff), CTSU staff

1. Primary responsibility for the following activities, for example:
    a. Determining eligibility: Eleanor Leavens, Leah Lambart, Stephanie Hiebert, Leo Byer, Nikki Nollen, Terri Tapp
    b. Obtaining informed consent: Eleanor Leavens, Leah Lambart, Stephanie Hiebert, Leo Byer, Terri Tapp

c. Providing on-going information to the study sponsor and the IRB: Eleanor Leavens, Dani Li, Leah Lambart, Stephanie Hiebert
d. Maintaining participant's research records: Eleanor Leavens, Tricia Snow, Matt Mayo, Leah Lambart, Stephanie Hiebert, Leo Byer
e. Completing physical examination: N/A
f. Taking vital signs, height, weight: CTSU staff
g. Drawing / collecting laboratory specimens: CTSU staff
h. Performing / conducting tests, procedures, interventions, questionnaires: Eleanor Leavens, Tricia Snow, Leah Lambart, Stephanie Hiebert, Leo Byer, CTSU staff, Terri Tapp
i. Completing study data forms: Eleanor Leavens, Leah Lambart, Stephanie Hiebert, Leo Byer
j. Managing study database: Matt Mayo, Tricia Snow, Eleanor Leavens, Nikki Nollen, Leah Lambart, Stephanie Hiebert, Leo Byer

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Elements of the plan include:
   The current study does not pose more than minimal risk; however, we are extremely sensitive to the history of cases of acute lung injury associated with e-cigarette products and the CDCs warnings. We continue to closely monitor the situation. To address this concern, we will obtain informed consent, closely monitor AEs and SAEs and promptly report any that occur. In addition, we will have firm stopping rules to protect the safety of study participants. The Vuse Alto EC will be used for the proposed study. The Vuse Alto device is a commercially available pod-based device and is compatible with nicotine salt and freebase nicotine e-liquid. We will use commercially available nicotine salt e-liquid in 5% and 1.8% nicotine concentrations.

2. All adverse events (AEs) occurring during the study will be assessed (e.g., symptoms checklist, NCI's Common Toxicity Criteria (CTC) version 5.0), documented, and reported to Drs. Leavens (Principal Investigator) and Nollen (Primary Mentor). The occurrence of AEs will be assessed each study visit throughout phase 1 and 2 of the study. Drs. Leavens and Nollen will review the AE logs at least weekly for all patients on the trial. The study investigators will follow all AEs to the point of a satisfactory resolution. All AEs will be assessed to determine if they meet criteria for an SAE. Serious adverse events (SAEs), as defined by the FDA, will be systematically evaluated at each visit. Any SAE, whether or not related to study, will be reported to the KUMC IRB, DSMC, and the FDA. The initial SAE report will be followed by submission of a completed SAE report to all three institutions.

3. In the event that a participant discontinues study treatment due to an SAE, the participant will continue to have appropriate follow-up and assessment. Outcome of SAEs

will be periodically reported to NIH. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NIH.

III. Subject Participation

A. Recruitment:

1. African American and White current daily smokers will be recruited from the local community using standard media outlets, combination of flyers, referrals, social media, and radio/TV ads, and in collaboration with Swope Health Center, which serves a large proportion of AAs and has been a recruitment site for all of Dr. Nollen's previous trials. We will also use the Frontiers registry, SlicerDicer, C3OD, and HERON databases to identify adult smokers who have agreed to be contacted for research. Consistent with past trials, we will send the patient a letter informing them of the study. We will then follow-up via phone the week after the letter was sent and offer screening. We will post the study to the KUMC Intranet list of current studies for KUMC employees. Additionally, participants are currently being screened for other research studies conducted by our team. Those who have completed studies or who are ineligible for other studies being conducted will be informed about the current study and offered the opportunity to be screened.
2. Recruitment methods are described above. Recruitment will be conducted by members of the study team. Recruitment will be overseen by Dr. Leavens. Screening will be conducted over the phone and by redcap survey.
3. Advertisements and flyers that will be used for recruitment will be submitted prior to study recruitment efforts begin. Advertisements and flyers will be handed out to potential participants by the study team and will be placed in clinics at KUMC/TUKHS and in the community.
4. Letters will be mailed directly to patients to inform them of the study. Within one week of mailing letters, study staff will make follow-up calls to patients and offer initial screening. Letters will be submitted prior to study recruitment efforts begin.

B. Screening Interview/questionnaire: The initial screen will review inclusion/exclusion criteria aside from exhaled carbon monoxide (eCO) measurement. Participants who are deemed to be eligible at the initial screening will report to the lab for in-person final screening within 14 days of the initial screening. During the final screening visit, participants will complete informed consent procedures, including HIPAA documentation. Only those participants who provide consent will complete the additional screening questionnaires. Additional screening measures include questionnaires, an expired carbon monoxide sample to confirm smoking status, and (if female) a urine pregnancy test. At the final screening and enrollment visit, participants will be asked to sample two Vuse EC flavors to determine flavor preference. Participants will complete baseline measures including demographics, medical, tobacco use, and substance use history and measurement of cotinine, NNAL, eCO, spirometry, blood pressure, lung injury panel, and respiratory symptoms.

C. Informed consent process and timing of obtaining of consent

1 Consent procedures will be conducted by trained members of the research team. Prior to consent, participants will be provided a detailed and comprehensive overview of study procedures.

2 Individuals interested in the proposed study and deemed to be initially eligible will meet the research assistant at the Clinical and Translational Science Unit (CTSU) at KUMC. Each individual will be given a copy of the consent form and as much time as they need to review its contents. After the consent form is read, both the individual and the research assistant will review the consent form together and the potential participant will

be encouraged to ask questions. Each individual will be reminded that participation in the study is completely voluntary. The consenting process will take place in a private location.

3 We do not anticipate recruitment of subjects with compromised cognitive abilities and/or decisional impairment. However, if questions regarding a participant's ability to provide informed consent arise, Dr. Leavens will determine whether the subject is able to give informed consent.

D. Alternatives to Participation: The alternative to participation is not participating, continuing to smoke cigarettes as usual, obtaining an EC on their own, attempting to quit using FDA-approved pharmacotherapy, attempting to quit cold turkey.

E. Costs to Subjects: There are no costs to participants. All tests, procedures, and visits are being performed solely for research purposes and are not billable to insurance companies.

F. How new information will be conveyed to the study subject and how it will be documented: We have plans to publish data from this study in aggregate but will not provide any individualized feedback to patients.

G. Payment, including a prorated plan for payment: In Phase 1, participants will receive $60 for each of the human laboratory visits. If they complete both human laboratory visits, they will receive a $20 bonus. Therefore, participants who attend screening and both study visits will earn $140. In Phase 2, participants will receive $20 for attending each randomized trial study visit/phone call which will be paid at the next in-person visit. In addition, they will receive $80 for providing samples and bringing their used and current e-cigarette pods for measurement. Participants who complete all Phase 2 procedures will earn $220. Participants who complete all study procedures can earn up to $360. Participants may also receive $20 for each referral who is eligible and enrolls in the study. They may complete up to 3 different referrals for an additional total of $60.

H. Payment for a research-related injury: N/A

IV. Data Collection and Protection

A. Data Management and Security:

1. We will use the participant's name only on the screening and informed consent/HIPPA documents and these will be kept in a locked file cabinet, to be kept in our study office. Protection against loss of confidentiality and privacy will be maintained by numerically coding all data, disguising identifying information, and keeping data locked in file drawers or in a secure, password-protected database. All biospecimen samples are deidentified and stored in a freezer at the CTSU. Names of participants will be kept separate from participant data. Only study research assistants and the PI will have the information that connects participant names and ID numbers. All electronic data will be numerically coded and stored in a password-protected database, on a password-protected computer in a secure research space. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication. No information will be stored locally on the laptop/tablet; all information will be stored securely in the secure data capture system. Only summaries of

group data will be reported in any publications or presentations, with no identification of individuals. Because identifiable information will be collected, participant privacy will be maintained throughout the duration of the study by adhering to the regulations set forth by the HIPAA Privacy Rule. More specifically, identifiable information will not be released without written authorization of the participant. Mobile devices will not be used for data collection or storage. Identifiable data will not be sent outside of KUMC.

B. Sample / Specimen Collection: Blood will be collected for plasma nicotine measurement during both study visits during Phase 1. Six blood draws (one needle stick) per participant per visit will occur and be processed and analyzed. At both visits, blood samples will be collected at -5 (baseline), 5 (post 10-puff bout), 7, 15 (beginning of ad lib session), 45, and 75 (post-session) minutes. Seven mL of blood will be collected during each blood draw (7 mL per draw x 6 draws per visit x 2 visits = 44 mL total during study). Samples will be analyzed according to standardized methods[84] by Dr. Na Zhang at KUMC. Blood will be analyzed for nicotine content to assess nicotine exposure during the session. All samples will be de-identified and labeled with a study identification number. Blood samples will be aliquoted into two separate vials. One will be analyzed for the current study and one will be placed in a biospecimen repository if participant provides consent for biorepository. Samples will be stored at the Bioanalytical Laboratory at the Clinical and Translational Science Unit (CTSU) at KUMC. Samples will be accessible only to members of the study team. Results from biomarker analyses will be de-identified and shared only with members of the research team. Any resulting publications will present the data in aggregate; individual participants will not be identified. Samples (blood/plasma and urine) will be disposed of one month after the final report is sent out to the Principal Investigator, unless participants agree to have their samples stored for future testing. For participants who agree to future testing, urine and blood (i.e., plasma) samples will be stored indefinitely.

C. Tissue Banking Considerations: For participants who agree to future testing, samples will be stored indefinitely. New markers of nicotine, EC, and carcinogen exposure are being discovered and the stored biological samples would be used for analysis of these new markers. All samples stored for future biomarker analyses will be de-identified and accessible only to members of the study team. Results from these analyses will be de-identified and shared only with members of the research team. Any resulting publications will present the data in aggregate; individual participants will not be identified.

D. Procedures to protect subject confidentiality: Confidentiality will be maintained by assigning each participant a study identification number and numerically coding all data. All biological samples and survey data will be labeled with the study identification number and never with the participants name or other identifiable information. The association of the ID-code and the participant's name will be kept by Dr. Leavens in a locked file cabinet and will only be accessible to members of the study team.

E. Quality Assurance / Monitoring: All data will be directly entered into our electronic data capture system (i.e., RedCap or CRIS) that contains edit checks to control the quality and completeness of data entry. Completeness of data entry will be automatically verified before each assessment is completed. The electronic data capture system is behind the KUMC secure firewall with role-based access that is HIPAA and human subjects compliant. There are no plans for ongoing third-party monitoring.

V. Data Analysis and Reporting

A. Statistical and Data Analysis: Data will be summarized using means and standard deviations, both overall and stratified (by race). To assess aim 1, we will conduct a 2x2 crossover study and utilize linear mixed effects models to assess differences in total inhaled volume between 1.8% and 5% nicotine concentration globally and by race. This technique allows us to handle repeated measures that are inherently correlated. Similar analyses will be done on consumption of e-liquid, plasma nicotine levels, craving and withdrawal. Period and carryover effects will be considered in the models. If warranted, based upon results from aim 1, we will examine race and its interaction with nicotine concentration in the linear mixed effects models for all outcomes. However, we have limited power to detect an interaction and do not anticipate a differential change between AAs and whites, thus we must see a differential effect (e.g., one race responds to one dose and the other race responds to the other dose). If the interaction term is significant, the interaction will be probed. For aim 2, we will obtain the number of participants who have completely substituted ECs for combustible cigarettes for each group (1.8% vs. 5%) and by race, calculate the corresponding proportion, assuming that if they fail to return, they did not have complete substitution, and calculate the 95% confidence interval for this proportion for each group independently. Given the small sample size, we do not expect to have the ability to obtain statistical difference, but we will have solid estimates on these proportions for each nicotine concentration group and will be able to calculate an effect size for future studies which can be designed to test a difference. Similarly, we will have estimates on proportion of dual cig-EC and cigarette only use. Finally, for aim 3, we will estimate the mean, standard deviation, and median for the change in e-liquid consumption and biomarkers for each nicotine concentration group. Given the sample size and distribution of the mean, we will either utilize the two-sample t-test or Wilcoxon rank sum test to test if there are differences between nicotine concentration groups. Primarily, we will calculate the potential effect size on these variables globally and by race. We will examine the rate of adverse events globally (experience any adverse event) and by event type for each nicotine concentration group and by race. Data will be transformed as appropriate. While we do not make any specific hypotheses regarding sex differences, we will consider sex as a covariate in all analyses. We expect to recruit equal numbers of males and females to explore sex as a moderator of our study outcomes in exploratory analyses. All analyses will be conducted at the 0.05 Type I error level. We will not adjust for multiplicity; Bonferroni or other adjustments would be too restrictive in this pilot study.

B. Outcome: The primary study endpoint is identifying the impact of e-liquid nicotine concentration on compensatory puffing, EC and cigarette use patterns, and resultant exposure to biomarkers of harm among AA and white smokers. Our central hypothesis is that, compared to the high nicotine concentration, while vaping the low nicotine concentration, users will engage in compensatory puffing, resulting in greater e-liquid consumption (Phase 1). Moreover, rates of dual use and continued smoking will be higher for the low (versus high) nicotine concentration and will result in greater exposure to toxicants (Phase 2).

C. Study results to participants: Study results will not be shared with research participants.

D. Publication Plan: We plan to publish results in appropriate tobacco and public health journals such as Addiction, Tobacco Control, Nicotine and Tobacco Research, etc. In addition, results will be presented at regional, national, and international conferences.

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