Protocol

Changes in Biomarkers Associated with Use of Electronic Cigarettes among African American Menthol and Nonmenthol Smokers

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Specific Aims

In this study, a 2-group randomized design will be used to evaluate changes in biomarkers associated with the use of e-cigarettes with and without nicotine among African American menthol and nonmenthol cigarette smokers. 240 smokers (all African American) will be randomized into one of two study groups including 1) Active e-cigarettes (containing nicotine), or 2) nicotine-free e-cigarette (containing 0mg nicotine). Within each study condition, participants will be allowed to select their preferred flavor (menthol or non-menthol) of electronic cigarettes after sampling both flavors.) Blocked randomization will be performed to ensure balance in the number of intervention assignments between the study groups (i.e., active e-cigarette versus nicotine-free e-cigarette). The power calculation for this study is based a two-sided two-sample equal-variance t-test on the primary outcome of NNAL (pmol/mg creatinine) at week 6 (active e-cig group vs. control e-cig group), and assumes an alpha of 0.05, and an NNAL standard deviation of 0.80. With a sample size of 200 completers, this study will have 80% power to detect a difference in NNAL between the active and placebo e-cig groups of at least 0.4 standard deviations at week 6 (mean difference of 0.32 pmol/mg creatinine).

All participants will be provided with e-cigarettes for *ad lib* use based upon the study randomization assignment. Follow-up assessments will be conducted at 2, 6, and 12 weeks post randomization. Primary outcomes will be changes in biomarkers, including total nicotine exposure (TNE) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Secondary outcomes will be the patterns of use of combustible cigarettes and electronic cigarettes including dual use.

Primary Aim: To compare the effect of electronic cigarettes with nicotine to electronic cigarettes without nicotine on NNAL (a biomarker related to the use of combustible cigarettes) among African American menthol and non-menthol smokers.

Primary Hypothesis: At 6 weeks post-enrollment, there will be a greater reduction in NNAL among participants using electronic cigarettes with nicotine compared to participants using electronic cigarettes without nicotine.

Secondary Aim: To compare the effect of electronic cigarettes with nicotine to electronic cigarettes without nicotine on smoking behavior (combustible cigarettes) among African American menthol and non-menthol smokers.

Secondary Hypothesis: At 6 weeks post-enrollment, there will be a greater reduction in use of combustible cigarettes among participants using electronic cigarettes with nicotine compared to participants using electronic cigarettes without nicotine.

Significance

Approximately 1 in 4 adult smokers in the U.S. smoke mentholated cigarettes.[1] However, there is substantial variation in the use of mentholated cigarettes.[1] Up to 80% of African American smokers use mentholated cigarettes compared to approximately 25% of non-Hispanic white smokers.[2] African

Americans also experience disproportionately high rates of lung cancer[2] and other smoking-related diseases.[3, 4] Studies by our research team[5-7] and others[8] have suggested that mentholated cigarettes are more addictive and associated with lower quit rates compared to non-mentholated cigarettes. However, not all studies have found this association.[9, 10] The pattern of use of mentholated cigarettes in Minnesota is consistent with national data in that 22% of smokers used mentholated cigarettes in 2010 and the rates of menthol use is highest among priority populations including African Americans, unemployed, and low-income smokers.

The Family Smoking Prevention and Tobacco Control Act enacted by the U.S. Congress in 2009 gave the Food and Drug Administration (FDA) the authority to regulate tobacco products. In July 2011, the FDA's Tobacco Products Scientific Advisory Committee (TPSAC) submitted its final report with a recommendation that "removal of menthol cigarettes from the marketplace would benefit public health in the U.S.". Although the FDA has yet to issue its final decision on the TPSAC report, one possible action could be a ban of mentholated cigarettes by the FDA from U.S. markets. A number of studies have examined what might happen if menthol cigarettes were to be banned from the U.S. markets. In a recent survey, 471 adolescents and adult smokers were asked a series of questions about how they might react if menthol cigarettes were banned.[11] Thirty-five percent of menthol smokers said they would quit smoking if menthol cigarettes were banned while 25% said they would "find a way to buy a menthol brand". In another study, researchers used data from the 2003 Tobacco Use Supplement to Current Population Survey to project the impact that a menthol ban would have on smoking prevalence and smoking attributable deaths.[12] Results showed that in a scenario in which 30% of menthol smokers quit and 30% of those who would have initiated as menthol smokers do not initiate, the relative reduction in smoking prevalence would be 9.7% overall and 24.8% for blacks by 2050. These studies suggest that a ban on menthol cigarettes could result in 3 million menthol smokers deciding to quit smoking, the majority of whom will be ethnic minorities and other at-risk groups. Even if menthol cigarettes were not banned, it is possible that menthol smokers seek other methods to obtain nicotine that might impact their use of a combustible cigarette product that might include the use of non-combustible nicotine product such as electronic cigarettes. However, there is no published about the pattern of use of e-cigarettes by menthol smokers in particular and African Americans in general.

Electronic cigarettes represent a dramatic new nicotine delivery technology and are an emerging health issue. Since their being introduced in the United States in 2007, e-cigarettes sales have been doubling annually and are projected to surpass the $2 billion mark in sales in 2013.[13, 14] Also, in some countries as many as a third of current or recent ex-cigarette smokers have tried e-cigarettes.[15-17] In the U.S., the most recent data from a representative national sample of adults reported that 4.2% of adults used e-cigarettes everyday, some days or rarely.[18] Given the increasing popularity of e-cigarettes, there is a compelling need to understand the pattern of use across various populations, characteristics of users, effects on nicotine and other toxicant exposure. This is even more important among minority and other at-risk populations including African Americans that experience

disproportionate burden of tobacco-related health effects. The proposed study is therefore designed to determine the biomarkers associated with the use of e-cigarettes with and without nicotine by African Americans smokers; and to determine the patterns of use of electronic cigarettes among African American smokers. This study has high potential to contribute significant new knowledge that could be invaluable for reducing the burden of tobacco-related health problems in Minnesota and beyond.

Furthermore, the distributors of e-cigarettes promote them as free of harmful substances claiming that e-cigarettes do not deliver toxic doses of nicotine.[13, 19] However, a recent report by an NIH Workshop on e-cigarettes[13] reported that analysis of aerosols from several brands of e-cigarettes revealed differences in their efficacy and consistency of nicotine aerosolization which is attributed to differences in device design including heating elements, cartridge size, and battery strength.[13] Also, recent studies have reported the presence of trace amounts of nitrosamines and other potentially toxic compounds.[19, 20] However, the levels of potentially toxic compounds in e-cigarette vapor are 9-450-fold lower than those in the smoke of combustible cigarettes.[13] Our research team recently completed a ClearWay-funded pilot study to determine product preferences of menthol cigarette smokers (n=53) when they were asked to stop smoking. Menthol smokers tested a number of products including nicotine replacement therapies (regular and mint-flavored nicotine gum) and electronic cigarettes (menthol and non-menthol brands) and ranked their product preferences. Menthol smokers overwhelmingly (75%) selected menthol e-cigarettes to help them during a cigarette abstinence phase. The proposed study builds on findings from our prior work by seeking to evaluate the patterns of use of electronic cigarettes among African American smokers and biomarkers associated with use of e-cigarettes.

Although the role of electronic cigarettes in tobacco control is controversial,[21, 22] there is paucity of data to inform the debate. Limited available data show that e-cigarettes are used by millions in the US and other countries.[23] In a 4-country survey[24] that included the USA, Canada, U.K. and Australia, respondents reported using e-cigarettes for a variety of reasons including in smoke free zones (85%), for reducing smoking (80%), because it is perceived to be less harmful (75%), or to quit smoking (70%). Other studies suggest that e-cigarettes are capable of delivering nicotine into the bloodstream and reducing withdrawal as effectively as NRTs.[25, 26] It is important to note, however, that while e-cigarette users may have reduced their cigarette smoking, smoking fewer cigarettes may not confer any real reduction in total nicotine exposure or in the risk of tobacco-related diseases.[27, 28] None of the studies described above reported data on the patterns of e-cigarette use by African Americans. Because African Americans are disproportionately impacted by tobacco-related diseases, data on the pattern of use of e-cigarettes among African Americans is critical. African Americans have also been shown to metabolize nicotine differently than whites. Studies are therefore needed to determine whether African American smokers who use e-cigarettes without the intent of quitting smoking reduce their exposure to nicotine and tobacco-specific carcinogens. Also, although there has been a lot of focus on African American menthol smokers, at least 20%-25% of African American smokers and 75%-

80% of white smokers smoke non-mentholated cigarettes.[1] Some studies have shown slightly better outcomes for AA menthol compared to nonmenthol smokers.[5-7] Studying AA menthol and nonmenthol smokers in the same study would provide the opportunity to evaluate potential differences in outcomes by menthol status in the pattern of use of e-cigarettes and biomarkers of exposure to e-cigarettes. The primary aim of the current application is to determine the biomarkers associated with the use of electronic cigarettes among African American menthol and nonmenthol smokers.. The study will also assess differences in patterns of use of e-cigarettes between African American menthol and nonmenthol smokers and e-cigarettes with and without nicotine.

Unfortunately, clinical studies assessing the efficacy of e-cigarette for smoking cessation per se cannot begin without an Investigational New Drug (IND) for a chosen e-cigarette from the FDA;[13] IND approval requires a level of product data and manufacturing documentation that is currently not available to researchers. Efforts by our research team as well as other researchers to obtain an IND approval from the FDA have been unsuccessful. In view of the barrier created by the current FDA policy requiring IND approval for e-cigarettes, it has therefore become necessary to amend the design of the proposed study from a treatment study to one that will assess the patterns of use of e-cigarettes and changes in biomarkers of exposure among African American smokers.

Because the primary outcome of the amended design is changes in biomarkers among smokers not intending to quit rather than smoking cessation, it is our belief that IND is not required. Under current FDA regulatory environment, the amended design represents the best possible science that uses a randomized controlled design to fill critical gap in the tobacco control literature regarding the patterns of use of e-cigarettes among African American smokers. The proposed study is informed by findings from our formative research which demonstrated an overwhelming preference for menthol e-cigarettes by African American menthol smokers and therefore represents an important step for developing an evidence base for about e-cigarette use in this priority population and its impact on potential harm.

Preliminary Evidence

Our research team recently completed a ClearWay-funded pilot study (n=53) to determine cessation treatment preferences of menthol cigarette smokers in which menthol smokers tested six products including four types of nicotine gum (2mg & 4mg plain; 2mg & 4mg mint-flavored) and two flavors of electronic cigarettes (16mg menthol and non-menthol). Study enrollment was completed in about six weeks. Participants were all African American menthol smokers, 53% male, mean age 45.5 (SD=11.5) years, smoked 14.2 CPD at baseline (SD=5.1).

Of the 53 smokers who completed product selection phase, 73.5% selected menthol e-cigarettes, 18.9% selected non-menthol e-cigarettes, and 7.5 % selected 2mg mint nicotine gum for smoking cessation. At the final week six follow-up visit (after two weeks of using selected product),

participants on average smoked 4.5 CPD; they reported using 8.7 e-cigarettes total for the 2-week cessation phase (<1 e-cig/day); and four participants had quit smoking tobacco cigarettes.

Findings from our formative research informed the proposed study in a number of ways including, 1) the formative research showed that African American menthol smokers will engage in a clinical research that includes the use of e-cigarettes. Given that recruitment of the 50 participants was completed in less than two months with minimal attrition, established the feasibility of recruitment for the proposed study; 2) African American menthol smokers overwhelmingly preferred menthol e-cigarettes compared to other tested products as a smoking cessation aid. This finding informed our choice of menthol e-cigarettes for the proposed study; and 3) given the absence of data on the patterns of use of e-cigarettes and the impact of use on biomarker of exposure, a natural step is for a study to evaluate the effects of e-cigarette on smoking behavior in this population

Innovation

The proposed study is innovative in that it will be the first to determine the biomarkers associated with the use of e-cigarettes among African American menthol and nonmenthol smokers, and to determine the pattern e-cigarette use among this priority population. In addition, this study will examine the role of nicotine in pattern of e-cigarette use and biomarkers of exposure.

Both nationally and in Minnesota, racial/ethnic minorities as well as other at risk groups (e.g. youth, low SES) smoke menthol cigarettes at disproportionately high rates compared to the general population. These groups also experience disproportionately higher rates of tobacco-related socio-economic and health consequences compared to the general population. The proposed research will examine patterns of use of e-cigarettes among smokers of combustible cigarettes in this priority population of smokers and the impact of these behaviors on biomarkers of exposure. Such information is critical to addressing use of tobacco products among at-risk smokers in Minnesota and nationally.

Approach

A total of 240 (all African American smokers) will be randomized 1:1 into one of two groups: nicotine-containing electronic cigarettes or nicotine-free electronic cigarettes. All participants will be provided with e-cigarettes by the study, based upon the study randomization assignment and be asked to use ad lib. Follow-up assessments will be conducted at 2, 6, and 12 weeks post randomization. Primary outcomes will be assessment of urinary biochemical markers NNAL (tobacco-specific carcinogen) and total nicotine exposure (not specific to tobacco) (TNE). Secondary outcomes will be the patterns of use of combustible cigarettes and e-cigarettes.

Inclusion criteria: 1) self-identify as African American or Black; 2) age 18 years or older; 3) smoke at least five cigarettes daily for the past year; 4) Willing to use e-cigarettes; 5) Good physical

health (no unstable medical or mental health condition); 6) no contraindications for e-cigarette use; and 7) does not have a set quit-date in the next 30 days.

Exclusion criteria: 1) Recent unstable or untreated psychiatric diagnosis including substance abuse, as determined by the DSM-IV criteria; 2) use in the past 30 days of e-cigarettes; 3) pregnant or nursing.

Recruitment**:** will utilize methods we have successfully used in previous studies with African American smokers[33-36] including flyers at African American community, clinic, and university sites and events, paid advertisement in targeted newspapers, radio, and email panels with predominantly large African American audience. We will also post advertisements on Facebook and create a study Facebook page. Lastly, we will send recruitment letters to Fairview patients using the Fairview Research Recruitment Mailing service. In order to complete this process we will develop a mailing list through CTSI. Fairview will receive the mailing list and then work with UMN Addressing and Mailing to send our recruitment letter along with a Fairview cover letter to potential participants. Potential participants will then contact the study coordinator if they are interested in participating.

Randomization Scheme: Randomization will occur at the individual level at the baseline assessment. The study statistician will provide the study coordinator with group assignments in sealed envelopes that will only be opened after study staff have confirmed eligibility and completed final consent. To ensure balance in the number of intervention assignments between the study groups (i.e., active e-cigarette versus nicotine-free e-cigarette),blocked randomization will be performed and the size and sequence of which will only be known to the statistician.

Study Procedures

Baseline/Randomization visit: At this visit the study's goal and procedures as well as participant responsibilities will be reviewed with each participant. Those who are interested will sign a consent form and be asked to complete an expired carbon monoxide assessment to verify smoking status. If expired carbon monoxide is <8ppm, then a urine sample will be collected and tested with urine strips for the presence of cotinine. If expired carbon monoxide is <8ppm and cotinine is not detected in the urine, the participant will be ineligible. Eligible participants will complete baseline assessments including demographics, tobacco use, and nicotine dependence measures. Participants will then be randomized to receive either active e-cigarettes (containing nicotine), or nicotine-free e-cigarette (containing 0mg nicotine). Within each study condition, participants will be allowed to select their preferred flavor (menthol or non-menthol) of electronic cigarettes. Prior to selection of their preferred flavor, participants will be allowed to sample both menthol and non-menthol flavored brands of e-cigarettes by taking a few puffs from both brands during the visit. Participants will receive enough supply of study products to last until their next appointment plus 7 days extra for scheduling flexibility. All participants will be provided verbal and written usage instructions for e-cigarettes. They will be instructed to bring all, used and unused, e-cigarettes to every visit for product count as one of the measures of adherence. Urine will also be collected to assess NNAL and TNE.

Weeks 2 and 6: Approximately three days before each of these visits, participants will be contacted by study staff through reminder card and by phone. At these visits participants will update their contact information and complete a brief survey (combustible cigarettes and e-cigarettes use, withdrawal symptoms, etc), take expired CO test, and receive more e-cigarettes (week 2 only) to last until their week 6 visit. Study staff will check and record if participant has been actively using their e-cigarettes and count returned e-cigarettes. Urine will also be collected at week 6 to assess NNAL and TNE.

Week 12: Participants will complete a survey over the phone.

Compensation: Participants will be compensated for their time and transportation to minimize attrition. Based on our experiences in previous studies participants will receive $40 at baseline and week 2, $50 at week 6 visit, and $20 at week 12, for a maximum of $150 per participant over 12 weeks.

Nicotine E-Cigarette Arm: Participants will receive an active (brand and dose TBD) e-cigarette with the flavor of their choice. Participants will each be dispensed a free rechargeable e-cigarette starter kit with the necessary accessories at the randomization visit plus enough supplies at this visit and the week 2 visit for a total supply of 6 weeks. Trouble shooting support will be provided as well as phone numbers for both technical and medical assistance related to the use of e-cigarettes. Follow-up visit schedule and all other study protocol for participants in each arm will be the same. Although the study will only provide participants with e-cigarettes for 6 weeks, they will be told that they are allowed to purchase their own if needed after 6 weeks.

Nicotine-free E-cigarette arm: Participants will receive e-cigarettes containing 0mg nicotine with the same protocol described for the active e-cigarette arm above.

Human Subjects Protection:

This study will enroll 240 smokers, all African American. Inclusion and exclusion criteria have been discussed earlier in the proposal. Before any research is conducted, we will obtain IRB approval for the study protocol and informed consent documents. Data will be obtained exclusively for research purposes.

Potential Risks: The use of the e-cigarettes will be under supervision of Dr. Joseph. The e-cigarettes will be discontinued in patients who become pregnant or develop a contraindication. Patients will be told that e-cigarettes are not approved by FDA as a smoking cessation aid. Some studies have found that the levels of the toxicants in e-cigarettes are 9-450 times lower than in cigarette smoke and were, in many cases, comparable with trace amounts found in nicotine inhaler, an FDA-approved smoking cessation product.[37] Participants will be told even though there are no long term health impact data, there are some studies showing more immediate effects from e-cigarette use, e.g. decreased Fractional exhaled Nitric Oxide (FeNO), increased respiratory resistance and retardation of lung function,[38] and one case of lipoid pneumonia.[39] Another study reported that, exposure to propylene glycol mist for 1 minute was associated with decrease in a person's tear film stability, increase in ocular and throat symptoms, and slight reduction in forced expiratory volume in 1

second/forced vital capacity (FEV1/FVC), and increase in self-rated severity of dyspnea.[40] We will follow the NIH guidelines for reporting adverse events to the Institutional Review Board. Any problems needing medical attention will be referred to Dr. Joseph and the participant's healthcare provider.

Data Collection (Measures):

Due to the literacy level of the target population, if needed all questionnaire items will be read to, or along with participants, by a trained research assistant.[41] A number of these measures were used in our pilot project and other studies with African American smokers.

Outcomes

Cigarette Smoking Behavior. Use of combustible cigarettes will be characterized in a variety of ways. These include, for example, smoking status; number of cigarettes smoked per day, per week, and per month; smoking history (age when first smoked); quitting history; and cigarette brand smoked (menthol vs. nonmenthol). These questions have established validity and reliability, and have been used in a sample comparable to the one planned for the current study.[5, 7] Use of e-cigarettes will be assessed at baseline and weeks 2, 6, and 12 using one item from the Penn State Electronic Cigarette Dependence Index[42] which asks the participant, "How many times per day do you usually use your electronic cigarette?" (assume that one "time" consists of around 15 puffs or lasts around 10 minutes); scoring: 0-4 times/day = 0, 5-9 = 1, 10-14 = 2, 15-19 = 3, 20-29 = 4, 30+ = 5). Total NNAL can be used to validate the use of only e-cigarettes because NNK is not present in the fluids used in e-cigarettes but is present in tobacco products. We will calculate total NNAL/total cotinine ratio. If no other tobacco is used, then the ratio should be low. Dr. Hatsukami has extensive experience in the use of NNAL to validate tobacco use status.

Demographics: The demographic data that will be collected in the study includes housing, gender, education, marital status, age, income, and employment status.

Nicotine Dependence: We will assess using the 1-item Time to First Cigarette question taken from the Fagerstrom Test for Nicotine Dependence (FTND) to assess dependence to combustible cigarettes.[43] Dependence on e-cigarettes will be assessed at weeks 2, 6, and 12 using the 10-item Penn State Electronic Cigarette Dependence Index.[42] This scale has been validated with a large sample of ex-smokers and e-cigarette users.

Withdrawal Symptoms: We will use the modified scale developed by Hughes and Hatsukami.[44] Items in the scale include desire to smoke, irritability (anger, frustration), anxiety (nervousness), difficulty concentrating, restlessness (impatience), hunger, depression, and awakening. Responses will be summed to create a withdrawal severity index and used as covariates in our analyses.

Readiness to quit: We will use the 1-item Contemplation Ladder,[45, 46] an 11-point Likert-type scale with 10 rungs, and is anchored at every point.

Other substance abuse: Questions to assess alcohol and other substances will be derived from National Household Survey on Drug Abuse.[47] These measures will be used as moderators of intervention components.

Biomarker assessment: The primary biomarkers that will be evaluated include urinary total NNAL and total nicotine equivalent (TNE). The urinary biomarkers will be assessed at baseline and week 6. We will also assess expired carbon monoxide (CO) using a hand held, portable CO monitor (Bedfont Micro Smokerlyzer, Kent, England). We will use 8 ppm as the cut-off to verify smoking status as recommended by the SRNT Subcommittee on Biochemical Verification.[48] Our group has extensive experience with biochemical verification of smoking.[7, 33, 35, 49] Expired CO will be measured at all in-person visits. If expired CO is <8ppm, a urine test strip will be used to detect the presence of nicotine/cotinine. We will also calculate total NNAL/total cotinine ratio. The TNE and NNAL analysis will be conducted by the lab of Dr. Sharon Murphy at the University of Minnesota.

Table 1.
Procedures
Summary

Visit/contact	Screening	1	2	3	4
Time	7-14 days	Baseline/ Randomization	Week	Week	Week
	before Week 0	Week 0	2	6	12 (by phone)
Visit Duration (Minutes)		75	45	45	30
Procedures					
Consent, Pregnancy test*		X			
Weight, Height		X		X	
Expired Carbon Monoxide		X	X	X	
NicAlert (if CO is <8ppm)		X			
Intervention					
Active/Placebo E-Cigarettes administered		X	X		
Outcomes					
Biomarkers (TNE, NNAL)		X		X	
E-cigarette count			X	X	X
Cigarettes per day	X	X	X	X	X
Timeline follow back		X	X	X	
Predictors					
Demographics	X	X			
Smoking History	X	X	X	X	X
Nicotine withdrawal		X	X	X	
Nicotine dependence			X	X	X
Readiness to quit smoking	X	X	X	X	X
Product evaluation			X	X	X

Rationale for Study Design

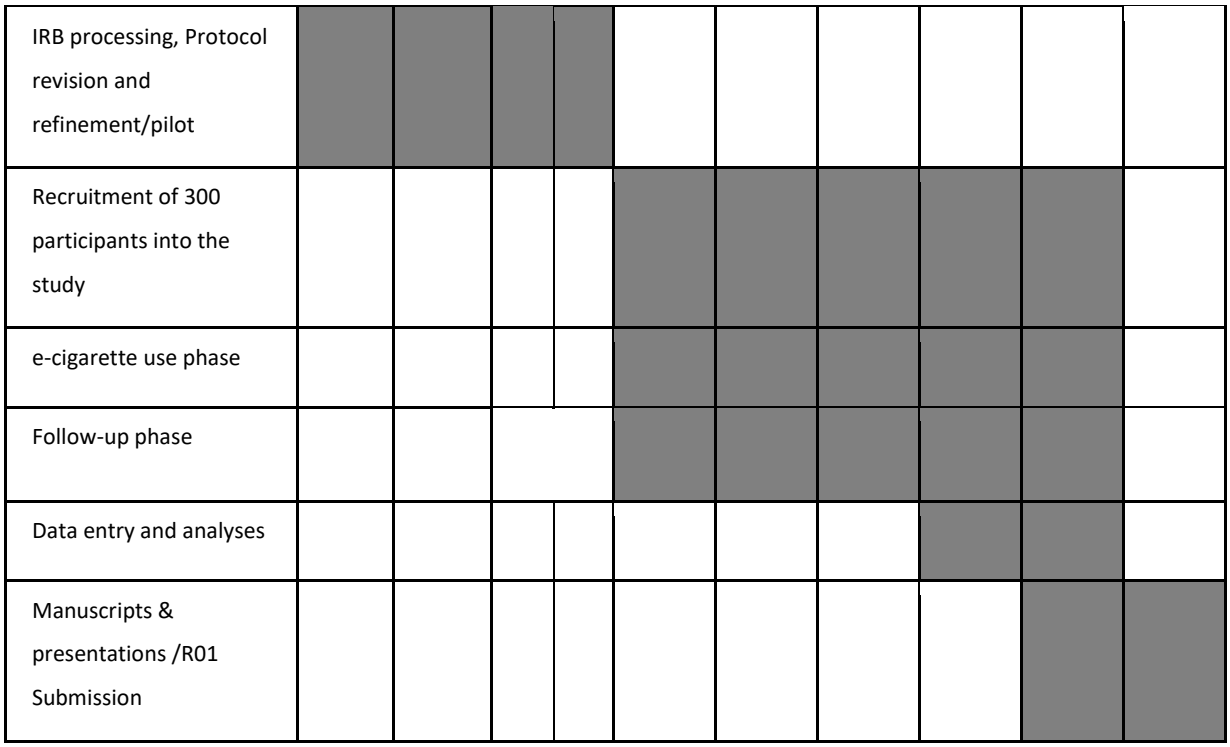
1) Overall design: The current study design was informed by findings from our formative study that showed that African American menthol smokers overwhelmingly selected menthol e-cigarette for smoking cessation. Using a randomized design would reduce potential differences in smoking behavior (e.g. CPD, motivation and confidence to quit, quit attempts, psychosocial factors, etc) between study groups that might impact the outcomes of interest. A 2-group randomized design will be used to evaluate the patterns of use of e-cigarette and the impact of use on biomarkers of exposure.

2) How was the brand and dose of e-cigarette selected? In our recently completed pilot study, we used the Blu e-cigarette (approximately 13-16mg nicotine) which is among the most commonly used and widely available e-cigarettes. The product was well-liked and well-tolerated by participants. However, there are more recent generations of e-cigarettes that may deliver a higher amount of nicotine. Therefore, if this proposal is funded, we will review all available options in the market and consider factors such amount of nicotine delivered, price, and affiliation of manufacturer with tobacco industry in final product selection with guidance from Dr. Hatsukami, an e-cigarette expert on our team.

3) Why limit study sample to African Americans? There are well-documented differences in smoking patterns and nicotine metabolism between African Americans (AAs) and whites. These include the fact that AAs smoke fewer cigarettes per day and predominantly smoke menthol cigarettes.[29] AAs also have higher cotinine levels per cigarette smoked than whites.[30] Differences in smoking patterns and nicotine metabolism in specific populations could introduce variability that masks potential differences in how smokers use e-cigarettes effects. Also, in the TPSAC report to the FDA, the population that had lower success rates for smoking cessation among menthol smokers compared to non-menthol smokers was predominantly African American (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/TobaccoProductsScientificAdvisoryCommittee/UCM269697.pdf).

Table 2. Project Timeline

	Year 1			Year 2			Year 3		
Months:	1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36



Power Calculation

The power calculation for this study is based a two-sided two-sample equal-variance t-test on the primary outcome of NNAL (pmol/mg creatinine) at week 6 (active e-cig group vs. control e-cig group), and assumes an alpha of 0.05, and an NNAL standard deviation of 0.80.[50] With a sample size of 200 completers, this study will have 80% power to detect a difference in NNAL between the active and placebo e-cig groups of at least 0.4 standard deviations at week 6 (mean difference of 0.32 pmol/mg creatinine). This is appropriate given a prior study with similar methods saw a decrease in NNAL of approximately 0.3 pmol/mg from baseline to week 6 among smokers that switched to reduced nicotine cigarettes (CENIC, n=196, p=0.02).[51]

For example, if the control e-cig group has no reduction in NNAL from baseline to week 6 (i.e. 1.50 to 1.50 pmol/mg creatinine), this study will be powered to detect a difference between study groups with at least a 21% reduction in NNAL in the active e-cig group (i.e. 1.50 to 1.18 pmol/mg creatinine). When comparing expected NNAL at week 6 between the two groups (active: 1.18 vs. control: 1.50 pmol/mg creatinine), the NNAL ratio is 0.79. This is comparable to the results in CENIC, which found a statistically significant NNAL ratio of 0.78 comparing participants that used 15mg/g cigarettes vs. participants that used reduced nicotine cigarettes (0.4mg/g) at week 6 (CENIC, n=196, p=0.02; Donny et al. 2015). To ensure that we have 200 participants complete the week 6 visit, we will inflate the randomization number by 20%, to 240 randomized, or 120 per group. This power calculation was completed using SAS Software v.9.4 (SAS Institute Inc., Cary, NC).

Analysis Plan

Prior to initiating outcome analyses of quantitative data, we will examine frequency distributions for all variables, with particular attention to variable ranges, missing values, and skewness, and will log-transform variables as necessary. Using t-tests for continuous data and chi-square tests for categorical data, we will examine randomization group comparability at baseline to determine whether randomization was successful in creating equivalent groups with regard to the demographic variables and baseline characteristics (i.e. age, gender, cigarettes smoked per day, nicotine dependence, and menthol vs. regular cigarette smoker). These analyses, along with a priori reasoning will help determine whether there is a need to incorporate covariates into later analyses.

Primary analyses: The primary outcome for the study will be NNAL at week 6. We will compare week 6 NNAL between the two study groups, adjusted for baseline NNAL in generalized linear regression models. Covariates that will be considered include age, sex, income or educational level, and menthol status. To test for a within-subject change in NNAL from baseline to week 6, paired t-tests will be used within randomization group.

Secondary analyses: Differences in our secondary measures between the two study groups will be assessed using the appropriate methods (e.g., logistic regression for binary and binomial outcomes, linear regression for continuous outcomes; using their Generalized Estimating Equations or mixed model analogs when the outcomes were repeatedly measured within person). Specifically, differences in smoking behavior between the two groups will be assessed by comparing the change in average number of cigarettes per day on the 7 days preceding the baseline visit to each study time point (week 2, week 6, and week 12). Other secondary measures that will be compared between the two groups may include: change in TNE from baseline to follow up visits, e-cigarette use from baseline to week 6, e-cigarette use from week 6 to week 12, and e-cigarette product evaluation.

Data Management: Data management activities for this project will encompass data entry, data cleaning, identifying and tagging any crossovers, conversion into proper format for data analysis and recoding. REDCap will be used for designing, implementation and maintenance of the database. In addition, a computer based tracking system will be developed to follow each patient and to prompt the staff for the upcoming data collection point. Data collection points for each subject will be calculated from his or her initial date of contact. Data entry will be performed at the University of Minnesota. Codebooks will be developed and will include variable formats (numeric/alpha), min/max ranges and any skip patterns.

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