Study Title: "Examination of First and Second Generation E-cigarettes" (NCT03113136)

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Examination of First and Second Generation E-cigarettes

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Revision History:

Revision Summary	**Protocol Version date**	**Consent Form Version Date**	**IRB Approval Date**
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Abstract

The 2009 Family Smoking Prevention and Tobacco Control Act grants the FDA regulatory authority over tobacco-based products. However, the FDA only recently proposed deeming regulations on e-cigarettes. Since their market emergence, e-cigarettes have evolved from first generation products, which look like a conventional cigarette, have few design components and limited nicotine delivery, to second generation products, which do not look like conventional cigarettes, have multiple design components and can achieve cigarette-like levels of nicotine-delivery. Missing from the current literature is a prospective study assessing differences between these two major types of e-cigarettes on smoking behaviors and patterns, nicotine dependence, and on biomarkers of toxicant exposure and effect. Understanding the differential impact of first versus second generation e-cigarettes on smoking as well as measures of proximal health outcomes will help immediately inform the science base needed for the FDA to impose appropriate product specific regulations. The overall aim of the proposed study is to evaluate the effect of switching from conventional cigarettes to either a first or second generation e-cigarette on smoking behavior, product use patterns and continued use, as well as biomarkers of toxicant exposure and effects. To do so, we will randomly assign 453 adult smokers, naïve to e-cigarette use, to low-wattage devices (LWD), high-wattage devices (HWD), or usual brand cigarette control (UBC). All products will be provided for 12 weeks. Enrollment will be restricted only to smokers not planning to quit in the next 3 months. Prior to randomization, all participants will take part in a lead-in period to assess normal smoking behavior and to allow for stratification on important variables. Follow-ups will occur at 1, 4, 8, 12, 26, and 52 weeks. Our specific aims are to 1) assess the effect of provision of LWD vs. HWD on product switching, abuse liability, number of cigarettes smoked, and perceived nicotine dependence; 2) evaluate changes in biomarkers of harmful tobacco constituent exposure among participants assigned to LWD, HWD, and UBC; and 3) evaluate changes in biomarkers of toxicant effect among participants assigned to LWD, HWD, and UBC. This innovative study in its comprehensive examination of all e-cigarette devices as well as their impact on smoking behavior and health outcomes, will provide the scientific foundation the FDA and other agencies need to establish effective regulatory strategies for the manufacture, distribution, and marketing of e-cigarettes.

Project Narrative/relevance to public health

E-cigarettes are proliferating and evolving rapidly in the current unregulated market. To date, it is not clear how e-cigarette products in general will impact smoking behavior and downstream health outcomes, let alone the differential effects between first versus second generation e-cigarette products. The proposed work will prospectively examine the effect of switching to e-cigarettes on changes in smoking patterns, nicotine dependence, exposures to harmful toxicants, and downstream physiological health effects.

Potential Benefits of the Proposed Research

Whereas no assurance can be made to an individual participant that he/she will personally benefit from this research, the experience should be beneficial. All participants will be encouraged to quit smoking at the completion of the study and will be provided referrals to local cessation resources. Adequate protections are in place in the event of unlikely and mild risks for study participation. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

A. Specific Aims

The 2009 Family Smoking Prevention and Tobacco Control Act granted the FDA's Center for Tobacco Products regulatory authority over tobacco-based products. However, the FDA only recently proposed deeming regulations on e-cigarettes (ECs). Since their market emergence, ECs have evolved from low-wattage devices (LWD) which look like conventional cigarettes, have few design components and limited nicotine delivery, to high-wattage devices (HWD) which do not look like conventional cigarettes, have multiple design components and can achieve cigarette-like levels of nicotine-delivery. Each step of the evolution has led to increased consumer momentum, as well as increased demand from health officials to establish a science base to answer the question, "Are e-cigarettes a benefit or burden to public health?"
Our own as well as others' research is beginning to suggest that very few exclusive EC users use LWD products. However, for a majority of current HWD users, a LWD product was the first vapor-based product they tried. In other words, the limited available data suggests that LWDs may lead to dual use and/or serve as a gateway product to HWD products. HWD products, on the other hand, appear to be sufficient for some smokers for exclusive use—likely due, at least in part, to better nicotine delivery. Conversely, better nicotine delivery may also lead to continued long-term nicotine addiction. Moreover, HWDs may also yield more toxic and carcinogenic compounds per puff than LWDs due to higher heating of the vapor. Missing from the current literature is a prospective study assessing differences between these two major types of ECs on smoking behaviors and patterns, nicotine addiction, and on biomarkers of toxicant exposure and effect. Understanding the differential impact of LWD vs. HWD products on smoking as well as measures of proximal health outcomes will help immediately inform the science base needed for the FDA to impose appropriate product specific regulations.

The overall aim of the proposed study is to evaluate the effect of switching from conventional cigarette smoking to one of two types of ECs on smoking behavior, product use patterns and continued use, as well as biomarkers of toxicant exposure and effects.

To do so, we will randomly assign 453 adult smokers, naïve to EC use, to LWD electronic cigarette; b) HWD electronic cigarette; or c) usual brand cigarette control (UBC). All products will be provided at no cost to the participant for 3 months. Enrollment will be restricted only to smokers not planning to quit in the next 3 months. Prior to randomization, all participants will take part in a lead-in period to assess normal smoking behavior and to allow for stratification on important variables.

Aim 1:
To assess the effect of provision of LWD vs. HWD on product switching/substitution, abuse liability, number of cigarettes smoked, and perceived nicotine dependence. *Hypothesis 1a: Complete product substitution (vs. partial or no substitution) will occur at a significantly higher rate among smokers randomized to HWD products compared to those randomized to LWDs at 12, 26, and 52-week follow-ups. Hypothesis 1b: Compared to those randomized to UBC, LWD and HWD participants will smoke fewer cigarettes per day, have lower product abuse liability and lower perceived nicotine dependence.*

Aim 2: To evaluate changes in biomarkers of harmful tobacco constituent exposure among participants assigned to LWD, HWD, and UBC. *Hypothesis 2a: 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 to harmful constituents. Hypothesis 2b: Among those who completely substitute ECs for conventional cigarettes, LWD users (vs. HWD) will have significantly lower levels of biomarkers of exposure.*

Aim 3: To evaluate changes in biomarkers of toxicant effect among participants assigned to G1, G2, and UBC. *Hypothesis 3a: The degree of changes in biomarkers of effect (levels of DNA damage-markers indicative of increased cancer risk) will be directly associated with the degree of substitution—greater levels of substitution will confer a larger reduction in levels of DNA damage. Hypothesis 3b: Among those who completely substitute ECs for conventional cigarettes, LWD users (vs. HWD) will have significantly reduced markers indicative of increased cancer risk.*

Our team is uniquely suited to conduct this investigation. Understanding how smokers use ECs as they transition away from smoking and carefully examining their health effects is critically important. This novel study will contribute knowledge urgently needed by the FDA and health officials to effectively regulate ECs in a way that best serves public health.

B. Significance

The U.S. FDA and E-cigarettes

The passage of the 2009 Family Smoking Prevention and Tobacco Control Act (FSPTCA) grants the FDA Center for Tobacco Products (CTP) regulatory authority over cigarettes, cigarette tobacco, smokeless tobacco, and roll your own tobacco. This promising piece of legislation empowers the FDA to regulate the manufacturing, marketing, and distribution of tobacco products in order to reduce tobacco-related morbidity and mortality. E-cigarettes (ECs) do not currently fall under the FDA's regulatory authority; however, in April 2014, the FDA proposed deeming regulations of ECs as well as a call for additional scientific research to inform the development of effective EC regulation. The proposed regulations 1) ban the sale of ECs to minors, 2) ban vending machine sales, 3) require warnings that ECs contain nicotine, 5) require disclosure of ingredients, and 6) prohibit distribution of free samples but with exemptions for research purposes. These regulations are important first steps in effectively regulating ECs.

Innovation

No study has directly compared LWD vs. HWD in a prospective, long-term, randomized controlled trial. This study will break new ground and provide a rich data source for the FDA, other regulators and scientists to firmly understand the effect of EC use on both behavioral and health outcomes. Our study does not make the mistake of viewing all ECs as the same. Randomized controlled trials have almost exclusively examined LWDs and have found only modest effects on smoking behavior, while the majority of survey studies include a large portion of individuals using HWDs and generally find astounding effects on smoking behavior. Is this difference simply due to self-selection bias involved in the convenience sampling of survey studies and increased veracity of reporting in controlled trials, or are there truly differences? We believe there is a difference—some positive (higher probability of exclusive vs. dual use) and some negative (greater exposure to harmful and potentially harmful constituents and increased risk of continued nicotine dependence)—our study will be the first to directly evaluate these hypotheses.

The proposed study will also provide our team with enough time (12-months) to examine how EC use evolves: For those who slowly reduce their number of cigarettes per day, do they ever become exclusive users? Do those who become exclusive EC users stay exclusive? Do they move on to newer generation products? What is the time course for this evolution? Do they change from tobacco-flavors to non-tobacco flavors? Would they continue to use ECs if only tobacco flavors were allowed? What happens to their interest in one day quitting all nicotine? Our study will be the first to comprehensively examine these changes across the entire spectrum of EC products.

Our study will conduct the most comprehensive examination of changes in levels of cigarette and EC-related toxicants of exposure and how these levels vary between LWD vs. HWD users. We will examine exposure to tobacco-specific nitrosamines (TSNA), aldehydes, nicotine, and CO. Our study will also be the first to examine changes in the effect of toxicant exposure, specifically DNA damage (a biomarker for increased cancer risk), with a recently established and more sensitive measure of DNA analysis than any other used in previous tobacco research (PADDA, developed by Dr. Queimado); changes in lung functioning will be a secondary health outcome.

Overall, this innovative study in its comprehensive examination of all EC devices and measurement of behavioral and health outcomes, will provide the scientific foundation the FDA and other agencies need to establish effective regulatory strategies.

C. Approach and Preliminary Studies

Investigators

Dr. Theodore Wagener (PI) is Director of the Tobacco Research Center and a clinical psychologist. He received excellent training at Brown Medical School as a psychology resident and T32 postdoctoral fellow in cardiovascular behavioral medicine (NHLBI T32HL076134). He has continued this momentum over the last 6 years, gaining significant experience conducting state and locally-funded prospective, randomized control trials and survey studies examining non-cigarette, tobacco products including ECs[3,20,24,36]. Dr. Wagener has also published EC commentaries[37,38] and an EC review article[4]. He was the PI of NCI R21CA164521, investigating the provision of dissolvable tobacco vs. medicinal nicotine in the reduction of children's secondhand smoke exposure. He recently completed this study with excellent retention (84%) and several manuscripts are currently in preparation. Dr. Wagener has the experience, management and methodological skill, and knowledge necessary to successfully conduct the proposed study.

Dr. Laura Beebe (Co-I) has more than 21 years of experience conducting research and evaluation with tobacco control programs, with a focus on measuring addiction, tobacco cessation and prevention. She is the Principal Investigator for the Oklahoma site participating in the NCI-funded project, "Culturally-Tailored Smoking Cessation for American Indians," a collaboration with the Kansas University Medical Center. Dr. Beebe currently directs three internally funded e-cigarette studies. They include a feasibility study comparing e-cigarettes and traditional NRT effects on conventional cigarette behaviors among women with cervical dysplasia; a follow-up study of e-cigarette users calling the Oklahoma Tobacco HeIpline; and an analysis of 2013 Oklahoma BRFSS data which included 5 state-added questions related to e-cigarette use. She is well-suited to serve as a co-investigator on this transdisciplinary project, offering expertise in both epidemiologic methods and qualitative data collection and analysis.

Dr. Lurdes Queimado (Co-I) is an Associate Professor and the Director of Basic and Translational Research for the Department of Otorhinolaryngology (ORL) at OUHSC. She holds an endowed professorship, the Presbyterian Health Foundation Chair in ORL and has a 23-year history of conducting cancer research. Her expertise in head and neck oncology led to her contract as a consultant for the National Salivary Gland Tumor and Cell Line Biorepository. Dr. Queimado's work on DNA damage has resulted in several publications including: the development of a novel assay named PADDA to quantify DNA damage induced by low levels of genotoxic exposure[42], the documentation that PADDA is at least 10-fold more sensitive than other assays to detect DNA damage induced by even low levels of exposure to tobacco[43], and the documentation that DNA damage detected by PADDA is a potential biomarker for tobacco-associated head and neck cancer risk.[39] In

addition to serving as the subrecipient PI at OUHSC supervising follow-up visits, she will also provide medical oversight for the study.

Dr. Dorothy Hatsukami (Co-I) is a Professor at the University of Minnesota and has a 21-year history of conducting tobacco harm reduction and regulatory research. She is an internationally-recognized expert in these areas. She has a history of substantial funding from the NIH, including serving as the PI on a Transdisciplinary Tobacco Use Research Center, on a U19 (Models of Tobacco Product Regulation), and a U54 (New Standards for Nicotine in Cigarettes). She also previously served on the US FDA Tobacco Products Scientific Advisory Panel. Dr. Hatsukami has conducted numerous NIH-funded studies examining novel, non-cigarette tobacco products, including several switching studies like the current proposal. Dr. Hatsukami brings a depth and breadth of experience relevant to the proposed study.

Dr. Irina Stepanov (Co-I) is an Assistant Professor at the University of Minnesota and has a 20-year history of conducting research in the field tobacco carcinogenesis. She has conducted numerous studies analyzing TSNA and other toxicants in cigarettes, smokeless tobacco, and nicotine replacement therapy products. She also developed and applied new methodologies for the measurement of biomarkers, such as urinary metabolites and DNA adducts of human exposure to tobacco carcinogens. Dr. Stepanov is currently the PI of two NCI R01 grants. In one of these studies, she is developing a testing approach that can produce meaningful predictions of changes in human exposure due to changes in constituent levels in cigarette smoke. In the second study, she investigates endogenous formation of NNN from nornicotine in smokeless tobacco users. Dr. Stepanov brings analytical biochemistry expertise to the proposed study.

D. Research Design and Methods

Design Overview

We propose to conduct a three-arm, randomized, parallel group trial that will proceed in four phases over 12 months. Participants will access the screening questionnaire using a public survey link generated by REDCap. The survey link will be distributed on Facebook posts and ads, recruitment flyers and through OSU email distribution lists. During Baseline Phase 1 (-1), participants will attend an orientation and assessment visit and be asked to smoke their usual brand of cigarettes for 1 week. During Baseline Phase 2 (0), participants will be randomized to either LWD, HWD, or usual brand cigarette control (UBC). The LWD device provided will be an eCom-BT and the HWD device provided will be an Evolv DNA 250. At this visit, those randomized to an EC group will also be able to sample EC flavors and choose their preferred flavor for the next phase of the study. The Randomized Product Trial Phase (1-12) will last 12 weeks and smokers will be asked to attempt to completely switch to the product for the next 12 weeks; otherwise, directions for use will be ad libitum. Those randomized to UBC will be instructed to use the product ad libitum. All products, to which participants will be randomized, will be provided at no cost; UBCs will be provided based on the average amount used per week collected during baseline phase 1. The Surveillance Phase will last 40 weeks, during which time no product will be provided, only assessment of continued product use (or not) and other outcomes of interest (see Table 3), as well as biomarkers of toxicant exposure and effects will be measured. Over the course of the study, all participants will complete 8 study visits: -1, 0, +1, +4, +8, +12, +26, +52 weeks. Time points for study visits were based on matching other switching studies in the literature in terms of 12-week product provision[50] and 40-week surveillance period to allow sufficient time to examine any potential transitions[3,51]. Study visits will include completion of computerized surveys and collection of specimens for biomarker analysis. Participants will also be asked to

complete a brief (<2 minute) daily online smoking/product diary into a secure database via REDCap for the 12 weeks of product provision and for the week prior to the 26- and 52-week visits.

Study Procedures

Participants

Cigarette smokers will be recruited from advertisements through a variety of media outlets and the internet, including Study Search, as well as community events. Participants from other studies who have agreed to be contacted regarding other study opportunities will also be contacted. Staff from those studies will prepare contact letters/emails and call participants on behalf of this study. Participants interested will be referred to this study for screening. Though we will not oversample participants of any particular background, we will advertise across a variety of media to increase the likelihood of recruitment across a range of races, ethnicities, age, socioeconomic status and locations (rural/urban). Smokers will be able to complete an online screener questionnaire generated from a secure database to determine eligibility.

Participants who meet the following eligibility criteria will be contacted by phone and asked to attend the first baseline visit. Inclusion criteria: 1) smoke ≥5 cigarettes per day for the past year; 2) not currently engaged in a quit attempt ; 3) read, write, and speak in English; 4) report at least minimal interest in switching to an alternative product (> “not at all” on a Likert scale); 5) have not regularly used a tank system, mechanical mod, or advanced personal vaporizer EC, though previous use of cig-a-like devices of 10 or less days in the last month will be allowed; 6) plan to live in the local area for next year; 7) have access to a smart phone; 8) have access to email and 9) be 21 years of age or older. Exclusion Criteria: 1) <21 years old 2) unstable or significant medical condition such as respiratory, kidney, or liver disease that could potentially affect biomarker data; 3) unstable or significant psychiatric conditions (past and stable conditions will be allowed); 4) history of cardiac event or distress within the past 3 months;5) currently pregnant, planning to become pregnant, or breastfeeding (pregnancy and breastfeeding status will continue to be evaluated throughout the study at each visit); and 6) do not reside in the same household as a participant currently active (have not completed all study visits) in the study. If a participant is ineligible for the study at the time of screening, the participant can be reassessed at a later time to determine if they are now eligible i.e. A participant meets all other eligibility criteria, but is not eligible because they are currently pregnant or breastfeeding. The participant can submit a new screener after they are no longer pregnant or breastfeeding and can be reassessed for eligibility. Reassessment for eligibility will vary based on previous ineligibility criteria and will be determined on a participant by participant basis.

Recruitment Feasibility and Retention

Recruitment

We intend to recruit 453 cigarette smokers over a 40-month period (60 months – 4 months for prep – 12 months for final follow-up – 4 months for data cleaning/analysis). We have already recruited approximately half of the sample in Oklahoma (n=220); therefore, we plan to recruit an additional 233 participants from the Columbus, Ohio area. The adult population of the Columbus Metropolitan Area is ~2,078,725 (US Census estimate, 2017) and 22% of Columbus residents smoke. Given that EC use is expected to rise over the next several years we conservatively estimate an average rate of trying at 50% for the proposed study duration. Therefore, our pool of potential participants in our local area is 228,660 (2,078,725 X .22 X .50). Because we

are trying to enroll 453 participants (0.002% of our potential pool) over a 40-month period, we are confident that our recruitment approaches will yield sufficient numbers and in our ability to enroll ~11 smokers per month (453/40 months).

Retention

All participants will receive $5 per visit to offset travel costs except those who utilize Lyft transportation services provided at no cost by the research team to and from the study site for 8 visits ($40), receive $7, when applicable, for parking, $40 for visit 1 and $20 for each subsequent visit after that in which they attend ($180), an additional $20 for visits when specimens are collected ($100), $1 for each online diary entry ($105 possible) and an additional bonus up to $100 for protocol compliance (attending ≥ 7 visits and completing ≥70% of online daily diary entries, and returning study products) for a total up to $505. The bonus payment will be given at different time points to encourage participant engagement: $5 will be given at weeks 1, 4, 8, and 12 if the participant returns their study product for a total of $20 and the remaining $20 will go towards their bonus of completing their product phase at their week 12 visit. To receive the $5 that went towards the completion of the product phase at weeks 1, 4 and 8 visits, participants will need to attend the week 12 visit. The remaining $40 will be compensated at week 52 if the participant attended ≥ 7 visits and completed ≥70% of online daily diary entries. If an entire study visit is not able to be completed, the participant will be compensated $5 for travel and, if applicable, $7 for parking. We will also facilitate study visits by offering evening and weekend appointments as well as additional retention strategies (e.g., multiple sources of contact, reminder calls/texts/emails). To improve engagement and retention of study participants, participants will receive reminder calls in addition to email or text reminders. Reminders will be sent by text or email based on a participant's preferred method of contact. The participant's preferred method of contact for communication will be observed; however, alternative methods may be employed if issues arise with the preferred method (e.g., participant has run out of minutes; cellular providers are not compatible). These methods are consistent with our team's previous studies and have resulted in excellent retention rates.

Project Timeline

	Year 1			**Year 2**			**Year 3**			**Year 4**			**Year 5**		
Months	*1-4*	*5-8*	*9-12*	*1-4*	*5-8*	*9-12*	*1-4*	*5-8*	*9-12*	*1-4*	*5-8*	*9-12*	*1-4*	*5-8*	*9-12*
Preparation; supplies; training; IRB approval	X														
Recruitment & Enrollment		X	X	X	X	X	X	X	X	X	X				
Follow-ups		X	X	X	X	X	X	X	X	X	X	X	X	X	
Data analysis, report writing, dissemination															X

Detailed Study Procedures

Advertisements will direct interested potential participants to complete a secure, online screener. Information regarding eligibility and the participants' email addresses and phone numbers will be uploaded into a secure database. Potential participants will then be further screened by phone.

Phone Screening: Trained staff will provide a brief description of the study and for those still interested, schedule an orientation and assessment visit and provide directions to the study site. No cost ride services through Lyft will be discussed as an option for participants who may not have reliable means of transportation.

Orientation and Assessment Visit: During this first visit, participants will be informed of the study procedures and rationale and asked to provide informed consent. To ensure eligibility, participant smoking status will be biochemically confirmed via exhaled carbon monoxide testing (CO≥4ppm) and eligibility related questions will be asked again and responses verified. Pregnancy exclusion will also be confirmed with a urine test (n.b., pregnancy tests will be completed at each visit throughout the study). Participants will provide basic demographic and smoking history information consistent with questions used in the Tobacco Use Supplement to the 2006 Current Population Survey and PATH study[52] as well as additional standardized assessment questionnaires as described below and in Table 3. Participants will be instructed to smoke as usual until their Baseline 0 visit.

Baseline Phase 1 (usual brand cigarette): During this baseline period (1-3 weeks), we will collect online daily smoking diaries. Upon the participant's visit to the clinic after 1-3 weeks of usual use, we will collect additional subjective measures of use as well as collection of urine, blood, saliva, and oral cells for biomarker analysis; thus, we will establish participants' baseline smoking behavior in this phase.

Baseline Phase 2 (Randomization & Sampling): Immediately following the baseline assessment, participants will be randomized to one of three arms for a period of 12 weeks: 1) LWD EC, 2) HWD EC, or 3) usual brand cigarette control (UBC). Randomization will occur using a stratified block-randomization procedure with small, random-sized blocks. Randomization will be stratified by self-reported level of nicotine dependence, educational attainment, sex and age. Consistent with several of our recently completed and ongoing EC studies and to more closely mimic real-world use, those randomized to LWD or HWD will first sample five 0mg nicotine EC flavors (tobacco, mint/menthol, bakery/dessert, fruit, tobacco) to determine participants' flavor preference during the Randomized Product Trial Phase. Specific flavors across the six categories above will be determined with the most recently available data from our ongoing survey studies of EC users. After the participant selects a preferred flavor, the participant will then sample various concentration levels of the preferred e-liquid flavor. For LWD, concentrations sampled will be 6mg (extra low), 12mg (low), 18mg (medium) and 24mg (high) and for HWD, concentrations sampled will be 3mg (extra low), 6mg (low), 12mg (medium) and 18mg (high). For LWD, we still start nicotine concentration sampling at 18mg and for HWD, we will start nicotine concentration sampling at 12mg. If a participant prefers to not try a specific flavor and/or nicotine concentration, they can choose not to sample them. If a participant finds that the initial concentration (medium) they sample is not tolerable, we will not have the participant sample the highest nicotine concentration and ask them to sample the low and extra low nicotine concentrations. Participants will be asked demonstrate they know how to use the device after sampling procedures are completed. Consistent with our previous studies[20], participants will be instructed on how to use both the LWD or HWD products, provided a starter kit as well as ~30mL of e-liquid. The amount provided to participants will be documented and tracked throughout the study. Provision of refill cartridges or childproof bottles will be titrated up or down based on use. All products will be given to participants in their original packaging, with manufacturers' marketing, instructions, and recommendations for use and provided at no cost. We will supplement these instructions with additional information on storing the EC and all liquid and cartridges up and away from children and pets. Although overdose or accidental ingestion is very unlikely and has not occurred in Dr. Wagener's previous studies, all participants will be provided the state and national poison control telephone line as well as a "tip sheet" on recognizing signs of nicotine overdose.

Randomized Product Trial Phase: The purpose of this 12-week phase will be to assess the effect of provision of LWD vs. HWD on product uptake and use, smoking behavior, and nicotine dependence, as well as level of toxicant exposure and physiological effect. Participants randomized to LWD vs. HWD will be asked to attempt to completely switch to the product for the next 12 weeks; otherwise they will be instructed to use ad libitum. Those randomized to UBC will be instructed to use the product ad libitum. All products, to which participants will be randomized, will be provided at no cost; usual brand of cigarettes will be provided based on the average amount used per week collected during baseline phase 1. Follow-up study visits will occur at +1, +4, +8, and +12 weeks. At follow-ups, all unused product cartridges and refill bottles will be collected to account for reported use and a portion, in addition to the EC devices themselves, will also be used for subsequent HPHC analysis (see Biomarkers of Toxicant Exposure section). New cartridges and new liquid refill bottles will also be provided based on estimated use. Study visits will include completion of computerized questionnaires and collection of urine and exhaled breath samples for biomarker analysis (see Table 3 for measures collection schedule). All participants will also be asked to complete a brief (<2 minute) daily online smoking/product diary in a secure database via REDCap for the 12 weeks of product provision. Since e-liquid will no longer be provided at the end of the product trail phase, participants will be provided with a list of vape shops located in the Columbus, OH area.

Surveillance Phase: The purpose of this 40-week period is to assess how LWD vs. HWD use evolves over time; specifically, continued use of product assigned, transitions to other types of EC products, smoking behavior and patterns, as well as continued monitoring of biomarkers of toxicant exposure and effect over the long-term. During this phase, follow-up study visits will occur at +26 and +52 weeks, and will continue to include computerized questionnaires as well as collection of biomarkers. However, use of the daily online diary will be reduced to only the week prior the 26- and 52-week visits. Participants will be given atomizers throughout the enrollment period but will not be provided ECs, e-liquid or conventional cigarettes during this phase. Use of both will continue to be tracked. Those continuing to use any tobacco or EC product at the end of the surveillance phase will be strongly advised to quit smoking/vaping and provided the number to the Ohio Tobacco Helpline and other local smoking cessation resources.

Counseling

Counseling as to use or not use the product at follow-up visits will not be a part of this study. At the second visit, participants randomized to ECs will be asked to attempt to completely switch to the product for the next 3 months. In subsequent visits, we will continue to ask participants to completely switch/stay switched but will not provide additional counseling (e.g., coping skills) so as to not influence the natural outcomes of product use (or non-use). For example, we assume that for those who find the product unhelpful in managing cravings or otherwise unsatisfying they will move on to another product or return to smoking. In real world use, most who try EC will not have access to a trained therapist who can effectively help them problem-solve such issues. We will provide participants trouble-shooting tips for the mechanics of their product, provide replacement parts as needed, and also address adverse events as they arise (though <2% and mild in Dr. Wagener's previous EC studies), but will not offer ways (e.g., timing, puff duration, etc.) to make the EC a better substitute or its use a more satisfying experience.

Protocol Adherence and Quality Control

All research staff will have completed Human Subjects and HIPAA training. Standard operating procedures (SOP) will be developed and all staff will be trained to ensure adherence to the SOP. As is standard practice for our team's current studies, each visit will have its own checklist of specific measures to be completed and the order in which they are to be administered. To reduce data entry errors, we will use secure computer-based questionnaires for participants to complete, including daily diaries. All specimens collected for biomarker analysis will be given individualized logging codes for each participant. All key on-site personnel will meet face-to-face weekly throughout the entire study. Off-site investigators will also participate in these weekly meetings during the first year for project start-up; however, this will be reduced to every other week as the study progresses. During these meetings, recruitment, enrollment, data collection, data monitoring results, and any concerns or issues that may arise will be discussed. We will also plan for all key personnel to meet at the OSU site in the first year for start-up and then in the final year to discuss analyses, manuscripts, and future research based off of this study.

Measures

All measures, with the exception of the daily online diary, will be collected at the study site during the eight visits as indicated in Table 3. All measures, including daily diary, have been used previously by our team and are validated, unless otherwise noted. Biomarkers of exposure and effect were chosen because they have shown reasonable laboratory reproducibility, have clear differences in levels between smokers and nonsmokers, demonstrate a dose-response relationship, and/or decrease upon tobacco cessation[54,55].

Outcome Measures

The following outcome measures will be assessed during study visits or emailed to the study participant if they are not able to attend a visit: 1) Complete Substitution will be defined as ≥7 days of no more than 1 conventional cigarette smoked (assessed via the *Timeline Follow Back Questionnaire*[56] that assesses all tobacco/nicotine products used for prior 7 days), exhaled carbon monoxide of <4ppm (see biomarker measures below), and reported use of ECs over the same 7-day period; 2) EC Dependence will be assessed using an adapted version of the *Cigarette Dependence Scale*[57] and the *Penn State EC Dependence Scale*[88]; 3) Cigarette Dependence will be measured with the *Cigarette Dependence Scale*[57]; *4*) EC Likeability/Preference will be measured using items derived from the *Cigarette Evaluation Scale*[58] (7-point Likert-type scale assessing different dimensions of responses to EC (e.g., psychological reward, satisfaction, and averseness) and an EC adapted version of the *Product Evaluation Scale*[59]; 5) EC Abuse Liability will be assessed using both the *Drug Effects/Liking Questionnaire*[60] as well as the *Cigarette Purchase Task*[61,62] (modified to assess amount willing to pay across similar units of product, "How much would you be willing to pay for…a pack of your usual brand of cigarettes?…one e-cigarette cartridge?…1mL of e-liquid?"); 6) Transitions to other style of EC and tobacco products will be assessed by asking participants, "Have you tried any other e-cigarette devices since your last visit?" Affirmative responses will be followed-up with questions regarding type of product, frequency and duration of use; 7) Motivation Rulers: importance, confidence, and readiness to quit smoking and to quit all nicotine products (6-items total) will be collected to assess for changes in motivation over time[63].

All participants will complete a daily online diary of EC and/or conventional cigarette use during the Baseline and Randomized Product Trial Phases; during the Surveillance Phase, participants will be asked to complete daily diaries the week prior to their +26 and +52-week visit. E-mails or text messages, with a link to an online survey, will be sent automatically and participants will enter data directly into a secure database (REDCap) and to encourage completion, participants will be compensated for diary completion at their next clinic visit. The participant's preferred method of contact for receiving daily diaries will be observed;

however, alternative methods may be employed if issues arise with the preferred method (e.g., participant has run out of minutes; cellular providers are not compatible). If participants are unable to complete a daily diary using the link to the online survey, they may be contacted by other methods by Switch study staff to complete the daily diary. If participants become ineligible during the course of the study, compensation for daily diaries will be given only for diaries completed up to the date that they were deemed ineligible. Diary data will provide several outcome variables: 1) Smoking behavior: conventional cigarettes smoked per day and 2) EC use: amount of e-liquid use and frequency of EC use; and 3) EC flavor: report EC flavors used that day (categories for flavors along with examples will be provided.

Table 3. Outcome Measures	**Baseline**		**Randomized Product Trial**				**Surveillance**	
Weeks	**-1**	**0**	**1**	**4**	**8**	**12**	**26**	**52**
Self-Report Measures								
Sampling Procedures		X	X	X	X			
Product/Tobacco Use History and Exposure	X							
Demographic History	X							
Daily online e-cigarette and smoking diary	X	X	X	X	X	X	X	X
Timeline Follow-Back	X	X	X	X	X	X	X	X
Cigarette Dependence Scale 2			X	X	X	X	X	X
Cigarette Dependence Scale	X	X	X	X	X	X	X	X
Product Evaluation Scale			X	X	X	X		
Cigarette Evaluation Scale		X		X		X	X	X
Drug Effects/Liking Questionnaire (cigarettes and e-cigarettes)		X		X		X	X	X
Cigarette Purchase Task (cigarettes and e-cigarettes)			X	X	X	X		
E-cigarette product transitions questionnaire			X	X	X	X	X	X
Motivation Rulers (cigarettes and nicotine)	X	X	X	X	X	X	X	X
Biomarker Measures								
Total NNAL (urine)		X		X		X	X	X
Total NNN (urine)		X		X		X	X	X
N^6-HOMe-dAdo (blood)		X		X		X	X	X
N^2-ethylidene-dGuo (blood)		X		X		X	X	X
Total Nicotine Equivalents (urine)		X		X		X	X	X
Creatinine (urine)		X		X		X	X	X
q-PADDA (oral cells)		X		X		X	X	X
Exhaled breath CO	X	X	X	X	X	X	X	X
Pulmonary Function		X		X		X	X	X
Pregnancy Test	X	X	X	X	X	X	X	X
Saliva Sample		X		X		X	X	X

Biomarkers of Toxicant Exposure

At visits where specimens are collected, participants will complete the Biomarker modifier questionnaire (used extensively in Dr. Hatsukami's current biomarker studies, a measure of factors that may moderate biomarkers of exposure and effect) which includes items that examine: a) tobacco smoke exposure at

home, work and socially; b) frequency of alcohol and other drug use; c) perceived stress; d) recent respiratory symptoms; and e) current medications. Most biomarkers of exposure proposed in this study have been analyzed extensively in Dr. Stepanov's laboratory. Methods for the analyses are described in the literature, and Dr. Stepanov possesses the expertise and laboratory capacity to conduct these analyses. The collected urine samples will be aliquoted into 5-mL cryovials, and each vial will be labeled with a unique ID encoding the study, subject, sample collection time-point, and the type of assay. Aliquots will be frozen at -20 ºC until analyses are conducted. Saliva samples (up to 2-3 mL will be obtained to analyze for cotinine levels. We will also save additional aliquots for potential future analyses of biomarkers of exposure to other constituents identified in this Aim, or other biomarkers that may be developed over time. Blood samples will be collected into 'purple top' blood collection vacutainer tubes, and plasma, red blood cells, and buffy coat will be separated by centrifugation. Buffy coat (used to isolate DNA), plasma, and red blood cells will be stored at -80 ºC for potential future studies. Urinary total NNN and total NNAL. Exposure to NNK–an accepted cause of lung cancer in smokers[64]–is measured by urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1butanol (NNAL), which is the sum of NNAL and its glucuronides.[65] This biomarker has been widely applied in studies of smoking and cancer, and its relationship to lung cancer in smokers has been demonstrated.[66-68] Exposure to NNN, a well-established esophageal and oral carcinogen[69], is measured by the sum of urinary NNN and its glucuronide, referred to as total NNN.[70] Urinary NNN is strongly related to the incidence of esophageal cancer in smokers.[71] Only relatively small levels of NNN and NNK, have been reported to be present in EC fluids and aerosols.[27] However, according to both the published literature[72] and our preliminary data (Stepanov, unpublished), there can be significant variation in the content of these carcinogens in EC and potentially variations between G1 and G2 products. Total NNN and total NNAL will be analyzed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) using standard validated methods.[73-76] Briefly, urine samples will be mixed with stable isotope-labeled internal standards ([pyridine-D_4]NNN and [$^{13}C_6$]NNAL) and treated with β-glucuronidase to release free NNN and NNAL from their glucuronides. The samples will be further purified by solid-phase extraction and analyzed by LC-MS/MS monitoring transitions *m/z* 178 → 148 for NNN, *m/z* 184 → 154 for [$^{13}C_6$]NNN, *m/z* 210 → 93 NNAL and *m/z* 216 → 98 for [$^{13}C_6$]NNAL. Urinary HPMA. Acrolein exposure is measured by urinary 3-hydroxypropyl mercapturic acid (HPMA).[77] Acrolein was chosen because it is an intense irritant and lung toxicant, and a suggested lung carcinogen that is present in EC.[78] It will be analyzed using a validated method,[55] in which purified samples will be analyzed by LC-MS/MS in the APCI mode, monitoring *m/z* 220 → 91 for 2-HPMA and 3-HPMA, and *m/z* 223 → 91 for [D_3]3-HPMA (internal standard). *N*^{6}-HOMe-dAdo, and *N*^{2}-ethylidene-dGuo in leukocyte DNA. Exposure to formaldehyde and acetaldehyde can be measured via the analysis of *N*^{6}-hydroxymethyl-deoxyadenosine (*N*^{6}-HOMe-dAdo) and *N*^{2}-ethylidene-deoxyguanosine (*N*^{2}-ethylidene-dGuo), respectively, in human leukocyte DNA.[79,80]

Formaldehyde and acetaldehyde cause respiratory tumors in laboratory animals,[81,82] and were chosen in this study because of the reports of their presence in EC aerosol at varying levels.[27] These constituents are formed in EC aerosol via oxidation of humectants, such as glycerol and propylene glycol, which are present in EC fluids. This process depends on the heating voltage that is used in a given EC variety.[27] Thus, due to higher heating temperatures and variable voltage options, G2s may yield higher levels of aldehydes as compared to G1s. N^6-HOMe-dAdo and N^2-ethylidene-dGuo will be analyzed as described.[79,80] For N^6-HOMe-dAdo analysis, DNA isolated from buffy coat will be enzymatically hydrolyzed in the presence of $NaBH_3CN$ to convert the adduct to the more stable N^6-Me-dAdo. The hydrolysates will be further purified by solid-phase extraction, and analyzed by LC-MS/MS monitoring *m/z* 266 → 150 for N^6-Me-dAdo and *m/z* 271 → 155 for [$^{15}N_5$]N^6-Me-dAdo (internal standard).[21] For N^2-ethylidene-dGuo analysis, enzymatic hydrolysis of DNA will be also performed in the presence of $NaBH_3CN$ to convert the adduct to N^2-ethyl-dGuo; the hydrolysate will be purified by solid-phase extraction; and the purified sample will be analyzed by LCMS/MS monitoring *m/z* 296 → 180 for N^2-ethyl-dGuo and *m/z* 301 → 185 for [$^{15}N_5$]N^2-ethyl-dGuo (internal standard).[71,72] Urinary total nicotine equivalents. Total nicotine was chosen as a biomarker because it is the major known addictive constituent in tobacco and cigarette smoke,[83] and is the key ingredient in EC. Nicotine content can greatly influence the category of users to which a given EC variety will appeal, and also affect the extent and pattern of EC use, thus driving exposures to other constituents in the given product. Analysis of these biomarkers will be conducted by gas chromatography (GC)-MS, using standardized methods.[84-86] Urinary creatinine. Urinary creatinine will be analyzed to adjust biomarker levels for urine dilution. The analysis will be performed by Fairview-University Medical Center Diagnostic Laboratories (Minneapolis) with a Kodak Ektachem 500 chemistry analyzer. Exhaled Carbon monoxide (CO) will also be collected to corroborate self-reported complete substitution vs. not. *Note: in addition to human samples, Dr. Stepanov will also conduct comprehensive tobacco-related HPHC analysis on the vapor from a randomly selected subset of participant EC devices (10 from each arm). This serves two purposes: 1) to correlate constituent levels with biomarkers of exposure in study participants, and 2) to examine if any additional HPHCs should be included in our biomarker analysis.*

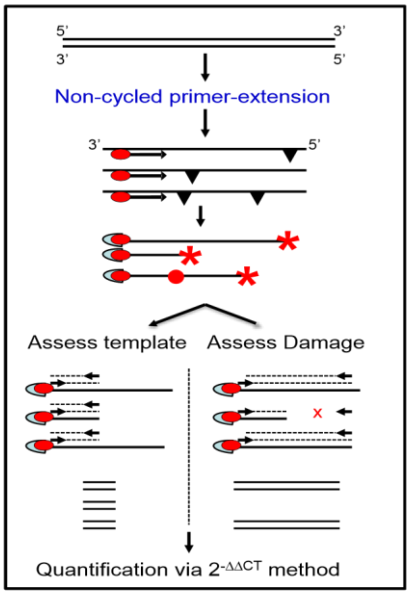

Fig. 6. Schematic representation of q-PADDA.

Biomarkers of Toxicant Effect (DNA Damage)

Oral cells will be collected using a soft brush (CytoPak CytoSoft Brush, Medical Packing Corporation, CA) [87]. Briefly, study subjects will be instructed to rinse their mouths with tap water, then brush and twirl each cytobrush for 30 seconds over the specified regions of the mouth. Areas designated for collection will be the top and bottom "gutter" areas (each with a separate CytoBrush), between the upper gum line and the mucosa of upper lip and cheek, and between the lower gum line and the mucosa of lower lip and cheek on both sides of the mouth. *DNA extraction* will be performed using standard methods as we previously described.[40,41] *DNA damage* will be quantified using q-PADDA (Fig. 6) in the transcribed strand (TS) and non-transcribed strand (NTS) of the *TP53 gene* (oligonucleotides listed in Supplementary Table 1; Appendix) as we previously described.[47,48] Briefly, a single non-cycled primer extension performed with a 5'-biotin-tagged primer and Vent exo- DNA polymerase identifies damaged nucleotides (Fig. 6, inverted triangles). The non-cycled primer extension generates a pool of highly specific biotin-tagged extended products (EP), each of them derived from one strand of a single DNA molecule. Each EP has a stop

(asterisk), which represents replicative arrest by a damaged nucleotide, or nick. After several purification steps, the extended single-stranded products will be re-quantification (q-PADDA).[47] Real-Time PCR reactions will be carried out in a CFX96 Real-Time PCR system in a 10 □l final volume with SsoAdvanced™ SYBR® Green Supermix (Bio-Rad), 0.3 □M of each oligo, and 1.5/100 of total extended product pool. The PCR conditions will be: 95□ C, 3 min; 40 cycles (95□ C, 12 sec; 62□ C for TS and 61□ C for NTS, 2 min). Samples will be amplified with two oligo sets for each strand-specific area to be analyzed. One set amplifies the targeted fragment (~ 700 bp) under analysis (damage template), while the other set amplifies a reference (~ 50 bp) immediately 5' to the primer used for the initial primer-extension (undamaged template). Each sample will be assayed in at least three independent experiments. To analyze differences in amplification efficiency between samples, a threshold value on early exponential phase will be used. Damage will be analyzed using the $2^{\square\square\square C}T$ method, or related to a standard curve for absolute quantification.[47,48] Lesion frequency will be estimated using the Poisson equation n=–ln(o).[47]
Other Biomarkers of Effect: Using a spirometer, a trained technician will measure Pulmonary functioning including, forced expiratory volume (FEV1), forced vital capacity (FVC), and FEV1/FVC.

E. Statistical Methods

Power Analysis

Complete substitution between LWD vs. HWD products is the primary outcome of the study, as rate of product substitution will likely drive the outcomes for all of the other aims; thus, we will estimate power for the proposed study based on this outcome at the 3-month follow-up. There are no direct data available on which we can base sample size estimates for the proposed study. The closest estimate for the LWD arm was based on the 3-month, 7-day point prevalence abstinence rate for the nicotinized EC (21.5%); however, as smokers in this study were motivated to quit smoking, set a quit date, and were provided cessation counseling, we conservatively estimate that the percent of complete substitution will be lower in the LWD group (15%). For the HWD group, we will assume a substitution rate of 30% based on the 2-month abstinence rate for HWD products found in the only prospective trial of HWD use[26]. As such, a total of 453 patients (151/group) will be enrolled into the study. This sample size will provide about 80% power to detect a 15% difference in complete substitution between LWD and HWD groups for a two-sided .05 level chi-squared test. In the sample size calculation, we assume the complete substitution rates are 15% and 30% for the LWD and HWD groups. Our estimates of sample size adjust for attrition rates of 20% in each arm. These attrition rates are a conservative estimate based on Dr. Wagener's previous research. We are confident that with a total sample size of 453 participants (n=363 after accounting for attrition), we will have adequate power to test our primary aim. It is important to note that this sample size is deliberately conservative, as it does not assume the availability of repeated outcome measures that will be taken throughout the study. By choosing models that utilize longitudinal data, we will be increasing the power to detect differences between arms.

Data Analytic Plan

Statistical analyses will be performed using SAS 9.2 or later. P-values less than .05 will be considered statistically significant. Baseline demographics and smoker characteristics will be summarized by groups (LWD, HWD, and UBC). Continuous variables will be presented as mean±SD and compared among 3 groups using ANOVA test. Categorical variables will be presented as counts and proportions and compared among 3 groups using the chi-squared test.

Aim 1: The focus of aim 1 is the examination of differential levels of (Hyp 1a) substitution between LWD and HWD, and (Hyp 1b) number of cigarettes smoked and nicotine dependence between G1, G2 and control. *Hypothesis 1a:* Complete substitution rates, at the end of the Product Trial Phase (3 months) and at both Surveillance Phase Visits (6 and 12 months), will be compared between LWD and HWD arms using multiple logistic regression models. If necessary, models will adjust for baseline variables such as age, gender, number of cigarettes per day and/or randomization stratification factors. The GEE model will be performed for the repeated measure analysis of complete substitution rate across multiple visits. *Hypothesis 1b:* If needed, we will apply a normalizing transformation to the response measure (e.g., taking the logarithm of cigarettes per day) before proceeding with the analysis, as a means of removing the effects of potential outliers. A single linear mixed effects regression model will be used to simultaneously estimate the product arm effects on outcomes (i.e., cigarettes per day, nicotine dependence, motivation to quit smoking/nicotine) at 1, 3, 6 and 12-month follow-ups, with a subject-specific intercept included to account for within-subject correlation in the outcome over time. We will control for baseline value of the outcome and potential confounders (including any variables not equally distributed between groups). Product arm will be coded using two dummy variables to account for the three product arms. Modeling is done using a likelihood-based approach and thus makes use of all available data (on the ITT sample) to produce consistent estimates of the regression parameters.

Aim 2: The focus of aim 2 is the examination of changes over time in biomarkers of toxicant exposure (Hyp 2a) between LWD, HWD and UBC, and (Hyp 2b) among LWD vs. HWD users who completely switch. *Hypothesis 2a:* The biomarker outcome measures analyzed will include mean levels of specific biomarkers at all follow-up time points. The analysis of biomarkers of toxicant exposure will be analyzed similar to outcome variables in Hypothesis 1b. Again mixed effects models will be used for analysis and resulting contrast estimates comparing group effects over time will be used as measure of effect. We will again apply a normalization transformation but will also include potential confounding factors measured in the *Biomarker Modifier Questionnaire* in the model. Hypothesis 2b: Similar analyses to Hypothesis 2a will be performed but only among the subset of LWD and HWD users who completely switch.

Aim 3: The focus of Aim 3 is the examination of changes over time in biomarkers of toxicant effect (Hyp 3a) between LWD, HWD and UBC, and (Hyp 3b) amongst LWD vs. HWD users who completely switch. For Aim 3 (Hypothesis 3a and IRB NUMBER: 6379 3b), we will run the same analyses as in Aim 2, but the biomarker outcome measures analyzed will include mean levels of DNA damage and pulmonary functioning at all follow-ups.

Missing Data

In the event of missing data, we will contact participants immediately. If a participant drops out, we will attempt to gather follow-up information. However, if they refuse to be contacted or otherwise lose contact with the investigators, we will censor data at point of loss. Two statistical approaches will be used to handle missing data. First, we will use inverse probability weighting with propensity scores. This is a two-step procedure in which we first model the probability of missingness as a function of baseline covariates and previous outcomes. Next, the inverse of the resulting predicted probabilities (from the logistic regression model) serve as weights in our proposed model of the response (e.g., salivary cotinine at follow-up). We will compare these results to a more conservative intent to treat approach as a final step.

F. Gender/Minority/Pediatric Inclusion for Research

Inclusion of Women and Minorities

According to 2018 US Census estimates, 51.3% of Columbus residents are female. We expect that the proportion of female participants will likely be somewhat larger given our previous studies with smokers (55-62% female). According to 2018 US Census estimates, the racial composition of individuals living in Columbus is 60.5% White, 28.3% Black or African American, 5.2% Asian, 0.2% American Indian/Alaska Native, 0.0% Native Hawaiian/Other Pacific Islander, and 4.1% two or more races. The ethnic composition of individuals living in Columbus is 6.0% Hispanic/Latino and 94.0% Non-Hispanic/Latino. We expect that our distribution will be similar to these but may potentially have a larger distribution of ethnic and racial minorities, given our previous studies and that menthol smokers tend to more often be black or Hispanic. However, we will continuously monitor enrollment in order to ensure that we are meeting recruitment goals to avoid under-recruiting minorities. If the targeted enrollment for minorities is not met because they do not respond to the advertisements, we will make special efforts to solicit their participation by advertising in community newspapers, local church organizations, and community centers.

Inclusion of Children

Participation in the proposed study will be restricted to individuals 21 years of age and older. This exclusion is for two primary reasons: 1) the use of tobacco products by minors is illegal, and 2) the concern of introducing and potentially addicting children and adolescents to another tobacco product.

G. Human Participants

Potential Risks

The research protocol calls for current smokers who do not plan to quit smoking within the next three months to attempt to completely substitute a LWD e-cigarette, a HWD e-cigarette, or to continue to smoke their usual brand of cigarette as they wish. Participants will not be provided any more instructions for use, and non-use of e-cigarettes is a permissible outcome. E-cigarettes are no more harmful than conventional cigarettes, and there is some evidence that they may offer reduced harm. Questionnaires, exhaled breath and urine collection procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) risk of using e-cigarettes, b) dual use of cigarettes and e-cigarettes, c) loss of confidentiality or privacy, d) potential for undermining smoking cessation, e) lack of appropriate storage of nicotine-containing products in a house with children and pets, f) slight risk of discomfort, bruising and infection with blood draw, and g) slight risk of discomfort and very low risk of infection with oral (buccal) cell collection.

The Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) and several states, and federal health departments are investigating a multi-state outbreak of severe lung disease associated with using e-cigarette/vaping products. The investigation is ongoing and has not identified a cause, but all cases have reported use of vaping devices. The majority of patients have reported vaping cannabis oil,

such as THC, obtained on the black market or other sources. In addition, one of the chemicals that has been possibly linked is Vitamin E acetate (tocopheryl acetate) added to e-liquid cartridges and pods.

The FDA has advised consumers to protect themselves by not using vaping products of any kind obtained off the street or black market and to refrain from using THC oil or modifying/adding any substance to vaping products purchased at stores. We will recommend that participants use only the products that we provide them for this study and do not add any substances to the e-liquids or alter the device in anyway.

Recruitment and Informed Consent

At first contact, all participants which include women and minorities will be screened according to the studies inclusion/exclusion criteria. Those who are eligible will be given a brief verbal overview of the study and invited to participate. Informed consent (including a description of the nature, purpose, risks, and benefits of the study) will take place through both oral and written explanation of the study. The voluntary nature of the study and the participant's right to withdraw at any time will be stressed during the consent process; a copy of the informed consent will be provided to the participant in written form at the time of consent for them to keep. Informed consent will be collected by IRB-approved study personnel. Recruitment scripts and materials, consent forms, and all study procedures will be approved by the OSU Institutional Review Board. All participants will provide written consent before any study data are collected.

Protections Against Risk

Efforts to reduce risk are as follows:

1. Risk of using e-cigarettes: The risk of side effects and adverse events are very low. These products are sold online, and at e-cigarette specialty stores and convenience stores nationwide, without a prescription. Nevertheless, all participants will be screened for general medical precautions (pregnancy, cardiovascular disease) and monitored for adverse events during the study period. Study personnel will assess for adverse events via self-report at all follow-up visits. Smokers will also be provided a study phone line to report an adverse event between follow-up visits. Any serious adverse events will be reported to the PI and then to the OSU IRB and potentially to the NIH. We will withdraw participants who have a serious adverse event, or become pregnant or begin to breastfeed. The most likely adverse (potential for nicotine overdose) event is anticipated to be rare (<5% in Dr. Wagener's previous studies) and mild (nausea, headache, disrupted sleep) event will be handled quickly (i.e., advice to participant to reduce or stop EC use). Dr. Queimado, a physician, will also be available for medical oversight and to handle any questions regarding reported adverse events. Lab studies of toxin exposure suggest that ECs incur no greater risk to health than do conventional cigarettes. Indeed, e-cigarettes generally show lower levels of harmful and potentially harmful constituents. To date, five e-cigarette studies discuss adverse events (3 survey and 2 randomized clinical trials), reporting mild and tolerable side effects that generally resolved completely over time with continued use (90% of cases); the most predominant of which were mouth/throat irritation, cough, and headache. In both randomized clinical trials, no serious adverse events were reported and the e-cigarette group and the nicotine patch group had comparable levels of adverse events. The most common were mouth irritation, throat irritation, dry cough and headache.

2. Risk of dual use of cigarettes and e-cigarettes: The concern of smokers engaging in dual use is that they will substantially increase their uptake of nicotine, leading to nicotine overdose. The symptoms of nicotine overdose include nausea, vomiting, dizziness, headache, and rapid heart rate. In our previous trial with e-cigarettes (see Preliminary Studies section), none of the participants reported any indication of nicotine overdose in their dual use of e-cigarettes and conventional cigarettes. In fact, most reduced their level of conventional cigarette use in proportion with their uptake of e-cigarettes. Preliminary analyses (n=20) from another one of our current randomized trials investigating the use of ECs by caregiver's as a means of reducing their children's SHSe (i.e., parents asked to use EC anytime they are in the home, car, or around their child), indicate that caregivers decreased in their level of salivary cotinine (Mbaseline=447.9 to M3-mo=314.8). Caregivers also reported reduction in number of tobacco cigarettes per day from baseline to 3-month follow-up (Mbaseline=19.6 to M3-mo=9.5). Consistent with these findings, parents reported no adverse events, no serious adverse events, and specifically, no nicotine overdose event.
3. Loss of Confidentiality and Privacy: Confidentiality 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 kept in a locked freezer and also will be deidentified. Names of participants will be kept separate from participant data. Only study research personnel and the PI will have the information that connects participants' 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 have completed training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication.
4. Potential for Undermining Cessation: The study sample is comprised of smokers with no plan to quit in the next three months. Therefore, we are not asking smokers who want to quit to continue smoking. Moreover, our previous study of e-cigarette sampling and use among smokers unmotivated to quit smoking suggested that e-cigarette use increased smokers' readiness and confidence to quit smoking. At the end of the study, all participants will be debriefed and educated about ECs and conventional cigarettes. This education will include the information that: a) there is no safe cigarette, b) the best thing a smoker can do to improve health is to quit, c) some ECs are manufactured by the tobacco industry, d) ECs, though able to be regulated by the FDA as a tobacco product, are currently unregulated by the FDA until they are able to develop appropriate guidelines (based on research such as the present proposal), and e) it is unclear whether ECs reduce the risks associated with smoking. The PI will be available for any questions that participants may have about ECs, smoking, or smoking cessation. It is important to note that the use of ECs incurs no greater harm than if the participant decided on his/her own to use the product. ECs are available online and over-the-counter at various convenience stores, e-cigarette specialty stores and places where tobacco products are sold. Among those screened and ineligible/uninterested, referral resources for smoking cessation will be provided for those who inquire. Among study participants, information on cessation resources will be provided at the final visit and if at any time during the study participants are interested in smoking cessation services, a list of smoking cessation resources will be provided.
5. Lack of Appropriate Storage of EC Products: Consistent with Dr. Wagener's previous EC studies, participants will be instructed to keep their UBCs, EC cartridges and childproof e-liquid refill containers up and away from their children and pets to protect against unintentional poisoning. Although overdose or accidental ingestion is very unlikely and has not occurred in Dr. Wagener's previous studies, all participants will be provided the state and national poison control telephone line as well as a "tip sheet" on recognizing signs of nicotine overdose. It is important to note that in the

unlikely event that someone other than the participant uses the EC, this risk would be no greater than if that same individual took the participant's cigarettes.

6. Slight risk of bruising, discomfort and infection with blood draw and oral (buccal) cell collection: Buccal cells and blood will be collected by trained research staff. Sterile instruments will be used and for blood draws, the participants skin will be cleaned with an alcohol wipe at the venipuncture site.

H. Data and Safety Monitoring Plan

Adverse events will be assessed by study staff at each follow-up visit via participant self-report and managed immediately. All adverse events will be reported to the OSU IRB. We will monitor for risk of smoking by screening participants for general medical precautions (pregnancy, cardiovascular disease). Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and Dr. Wagener. Dr. Wagener will be responsible for completing an Adverse Events Form should an event occur. Dr. Wagener will report potential unanticipated problems involving risks to subjects or others" (UPIRSOs) to the OSU IRB within 24 hours of having received notice of the event. Dr. Wagener will gather any information needed to investigate the event and to determine subsequent action. Any subsequent action will be documented and reported to the OSU IRB and the Program Officer at NIH. Adverse event reports will be reviewed annually with the OSU IRB to ensure participant safety.

Addendum

COVID-19 Related Procedures

Due to the COVID-19 pandemic, processes and procedures have been implemented to help protect participants and research staff. These processes and procedures are to be followed as long as social distancing requirements are necessary for conducting study visits.

Only one study participant per study coordinator will attend study visits at the CTR at any given time. All study participants will be provided with a face mask upon entry. Only one coordinator will meet the participant at their car for a temperature check**,** direct the participant into the building, and the two of them will ride the elevator to the 4th floor physically distanced at least 6 ft apart, both wearing masks. No more than 2 persons may ride the elevator at any given time. The participant will be immediately escorted to a private exam/draw room. Therefore, there will be no waiting in open lobby/waiting areas.

When in the exam room, the study coordinator will stand at least 6 feet away from the study participant to give instructions. Afterwards, the study coordinator will leave the exam room to allow the study participant to conduct the instructed procedures. The study coordinator and study participant will be at least 6 feet away from one another and wearing protective masks at all times during each visit.

Each study coordinator will have a designated exam/draw room and smoking room in which to conduct their designated research study. Each smoking room is separated from the staff control station in the hallway by its own door and contains a large window for the study coordinator to be able to see in and monitor study participant activity within the room. There is also a speaker and microphone system within each smoking room along with the Genetech software system on the outside of each room at the smoking room computer stations. Therefore, the study coordinator and study participant can communicate without being in the room together.

For study measures which cannot be physically distanced, appropriate PPE will be worn at all times by research staff during these procedures including goggles, face masks, gloves, and isolation gowns or lab coats.

Study surveys that are able to be completed remotely will be sent to participants using their preferred method of contact prior to the scheduled in-lab study visit. Surveys will be considered complete if at least 90% of survey responses recorded are valid. Repetitive use of “Refuse to Answer”, “0” or skipping of questions are not considered valid. Surveys completed remotely will be checked by research study staff. To receive the study visit portion of compensation, 90% of the survey responses recorded must be valid.

After each participant visit is complete, there will be at least a 45-minute period for cleaning and air exchanges in the negative pressure rooms and for cleaning exam rooms and equipment before the next participant visit. All smoking rooms are under negative pressure with a ventilation rate of 36.8 – 44.1 air changes per hour (ACH).

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