“Young Adult EC Use and Respiratory Outcomes (K01)”

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I. Objectives

Nearly 1 in 5 U.S. high school students reported current use of any tobacco product in 2017. Tobacco use is often started and established during adolescence,[15,16] with 90% of smokers starting before the age of 18.[15,16] Electronic cigarettes (ECs) remain the most commonly used tobacco product among young adults,[15-20] with new "pod mod" style devices, like JUUL, further driving up prevalence.[21-24] Studies in adults indicate that EC use is associated with decreased airway reactivity and inflammation, but no research has examined the effects of EC use on respiratory health in youth. Objective indicators of respiratory health (airway reactivity and inflammation) and pulmonary functioning are known predictors of lung disease (e.g., asthma[25,26], COPD[25,27-29]). As such, **prospective longitudinal research examining the impact of EC use on these indicators is vital to understanding the potential health consequences of EC use among young people.**

While national longitudinal studies, like the Population Assessment of Health and Tobacco (PATH), aim to capture tobacco initiation, perceptions, and patterns of use, it has several limitations. First, **PATH does not**

include objective measures of pulmonary functioning and respiratory health (e.g., airway reactivity and inflammation)—even though these factors are identified as an understudied and priority area of investigation by the American Thoracic Society (ATS)[30] and FDA[31]. Second, **PATH requires young adults to retrospectively report on tobacco use behaviors and does not link these behaviors to respiratory outcomes.** The present study will leverage multimethod approaches including biological and naturalistic measures (EMA) to innovatively capture pulmonary functioning and respiratory health prospectively among sample of young adult exclusive-EC users (*n* = 100) and never-users (*n* = 50) ages 18 – 25 years old. By collecting more granular detail of daily EC use patterns (e.g., frequency, quantity) and corresponding daily measures of pulmonary health, we will be able to more clearly determine the role of daily within-person fluctuations in frequency/quantity of EC use and its links to pulmonary functioning. Moreover, as cross-sectional surveys show that 54% of young tobacco users dual use ECs with OTP, we will also collect EMAs on experimentation with and co-use of other tobacco products (OTPs).[32,33] This data will provide an additional opportunity to examine the respiratory and pulmonary impact of dual and polytobacco use on respiratory health and pulmonary functioning among young adults.

The proposed study will address the current knowledge gap using a prospective, 1-year, longitudinal design combined with five intensive 2-week "measurement bursts" of EMA and multiple measurements of respiratory functioning. To examine the association between frequency of use (e.g., number of vaping sessions per day, frequency of days used), self-reported nicotine dependence, respiratory health, and biological markers of respiratory inflammation, complementary 2-week EMA and home-based spirometry (2/day, morning and night) assessments prior to laboratory visits will provide a more accurate estimation of young adults tobacco use as it occurs in, or near "real time" in the natural environment and the subsequent impact this has on acute reductions in pulmonary functioning. If never-users become users, we will incorporate relevant data into the user group at the visit during which they first report tobacco or EC use.

Aim 1: To compare longitudinal changes (over the 1-year monitoring period) in pulmonary functioning and respiratory health between exclusive-EC users and healthy controls in repeated sessions. *H1a:* Exclusive-EC users will have significantly lower levels of respiratory and pulmonary functioning (e.g., increase in inflammation markers (e.g., IL-6, IL-8); decreased forced expiratory volume (FEV1), forced vital capacity (FVC), FEV1/FVC ratio). *H1b:* Among exclusive EC users, greater frequency of use (number of vaping sessions per day, number of days used) and increased nicotine dependence (significant increases in dependence scores over 1 year) will be associated with decreased respiratory and pulmonary functioning.
Aim 2: To identify a dose-response relationship between quantity / frequency of EC use (daily EMA monitoring) and acute changes in pulmonary functioning (same-day, home-based spirometry). *H2a:* EC-exclusive users who report multiple vape sessions per day and spend more time vaping will show acute decreases in pulmonary functioning. *H2b:* Among all participants, use of ECs in combination with combustible OTP will be associated with decreased pulmonary functioning.
Exploratory Aim 3: **To identify the impact of EC+OTP use on respiratory and pulmonary health**. *H3a:* Dual users of EC+OTP will have decreased respiratory and pulmonary functioning compared to EC-exclusive and Never-tobacco users. We will examine how user status impacts acute (EMA/spirometry) and longitudinal changes in respiratory health (baseline to 12-month visit).

Understanding the respiratory health effects of e-cigarette use among young adults has implications for the development of tobacco use prevention campaigns and for the regulation of ECs by the FDA.

II. Background and Rationale

Public health efforts have been effective in reducing young adults use of cigarettes, and rates of cigarette use are the lowest ever recorded;[34] however, young adults use of ECs have rapidly increased and this may be changing how young adults engage with OTPs.[21-23,32,35,36] In 2017, an estimated 3 million youth (20%) reported current use of any tobacco product, and of those, 1.4 million reported current use of ≥2 tobacco products,[32] with a higher prevalence of use among males than females. **Among youth and young adults, ECs continue to be the most commonly used tobacco product** (11% of current users), followed by cigarettes (8%), cigars (8%), smokeless tobacco (6%), and hookahs (5%).[32,37] Moreover, the latest findings from the 2018 National Youth Tobacco Survey show **a 75% increase in EC use among high school students between 2017 and 2018**.[36] A handful of prospective survey studies suggest that EC use is associated with greater risk for subsequent cigarette

smoking and OTP use.[33,38] As such, public health officials and regulators are concerned that increased use of EC may lead to increased nicotine dependence and escalation of harmful tobacco product use.

Adolescence and young adulthood is a critical time in which tobacco product experimentation occurs, and recent studies highlight that EC experimentation has been shown to predict the initiation of combustible cigarette smoking and OTP use during this developmental period.[33,39-42] Data suggest EC use is less stable among young people), with significantly more transitions between various tobacco products.[43-45] This is concerning as multiple tobacco product use increases nicotine exposure[46-48] and may also increase nicotine dependence.[46-48] Nicotine is a highly addictive stimulant and dependence can be acquired even at low levels and prior to the initiation of daily use.[49-51] Thus, such fluctuations in young adults may be critical to the development of long term dependence. The biological consequences of nicotine dependence may be particularly harmful for young adults given the rapid changes in brain development during this time period.[16]

While the harmful pulmonary effects of combustible tobacco use are well documented, including COPD[25,27-29] and the development and exacerbation of asthma,[9,10,2527,52,53] very little is known about the respiratory effects of EC use. EC aerosol contains several chemical constituents such as diacetyl or benzaldehyde, known to affect the respiratory system.[54-59] To date, only a handful of studies have examined the respiratory effects of ECs in humans,[60-65] and none have focused on acute or longitudinal effects in young adults. These studies suggest that EC use has a significant impact on pulmonary and respiratory functioning.[60-66] Specifically, among adults, use of an EC for just 5 minutes increases airflow resistance,[64] decreases oxidative stress,[64,67] and increases particle deposition within the lungs – even more than cigarettes.[67] After 15 minutes of EC use, one study identified decreased cough sensitivity, but found full recovery within 24 hours of non-use.[68] Further, adult EC puff topography studies indicate that the average EC user inhales a significant volume of e-liquid aerosols,[69] which exposes the entire respiratory epithelium to substantial concentrations of fine particulate matter.[69] This is concerning as particulate matter can travel deeply into the respiratory tract into the lungs and cause health effects (e.g., decreased lung function, worsen medical conditions such as asthma and heart disease) upon inhalation.[69,70] Adult studies examining EC puff topography indicate that EC users' puff duration lasts significantly longer than a typical cigarette smokers' puff[71,72] (2.7-3.5 seconds[73,74] vs. 2.3 seconds[72]) and EC users have similar flow rates (about 37 ml/s).[74,75] This may potentially expose users to more chemical constituents (e.g., diacetyl or benzaldehyde[54-59]) and decrease pulmonary functioning and overall respiratory health.[58,76] These studies indicate that the respiratory physiologic effects of EC use, even after short-term use, are similar to some of the effects seen with tobacco smoking;[54-59,64,71,72,77] however, the long-term health effects of EC use are unknown, potentially adverse, and worthy of further investigation. Further, the current young adults literature is limited to respiratory effects derived from self-report[10,60,61] and cross-sectional[7,10,60] data. These data indicate a correlation between young adults EC use and chronic bronchitic symptoms[60] and increased cough or phlegm.[61] *Currently, no studies have objectively measured changes in respiratory health in adolescents as they relate to EC use, specifically, the impact of EC puff topography and frequency of use on respiratory and pulmonary functioning.*

This study uses complementary methods of data collection (self-report, biological samples, and EMA) to address several gaps and limitations in the extant literature. First, data from this study will complement current national longitudinal studies, like the Population Assessment of Health and Tobacco (PATH), National Youth Tobacco Survey (NYTS), National Survey on Drug Use and Health (NSDUH). While data from PATH, NYTS, and NSDUH capture youth tobacco initiation, perceptions, and patterns of use, *they do not include objective measures of pulmonary functioning and respiratory health* (e.g., airway reactivity and inflammation); important indicators for the development of asthma and COPD. **The present study addresses this knowledge gap by providing a comprehensive assessment regarding the acute and longitudinal respiratory effects of young adults EC use**. In the present proposal, each assessment (baseline, 3-, 6-, 9-, and 12-months) will employ a battery of well-validated, objective respiratory evaluations to examine how EC use is acutely related to respiratory inflammation and pulmonary functioning. Second, PATH, NYTS, and NSDUH require young adults to retrospectively report on tobacco use behaviors and do not link these behaviors to respiratory outcomes; outcomes that are correlated with tobacco-related health effects in older adulthood. **The present study will incorporate ecological momentary assessment (EMA) which will provide a more accurate estimation of the impact of EC use, measured in, or near "real time" and in the natural environment on pulmonary functioning. In addition**

to spirometry, this study will also incorporate secondary assessments of airway inflammation measured via nasal epithelial lining fluid (NELF) samples. If pulmonary functioning and respiratory health is found to be worse over time among young adults who use ECs compared to young adults who do not, this provides further evidence that ECs negatively impact public health and should be regulated more stringently by FDA. Additionally, the use of in-home and laboratory measures will provide more granular detail about how different EC use patterns (e.g., frequency, quantity) influence pulmonary health. While the PATH, NYTS, and NSDUH provide informative data regarding tobacco experimentation and initiation, perceptions of harm, and patterns of use, the retrospective nature requires young adults to report on behaviors over the previous year. We will also utilize EMA to obtain information about the experimentation with OTPs and elucidate the contextual factors associated with these behaviors. Finally, cross-sectional surveys estimate that among youth and young adult tobacco users, 42% use ECs exclusively [32], while 54% dual use ECs with OTPs.[32,33] This is particularly concerning as combustible tobacco use frequently leads to poor respiratory health, including smoking-related illness like COPD[25,27-29,78] and asthma.[25,27,53] **The present study will identify not only *when* experimentation and co-use of OTPs occurs, but the *impact of EC+OTP use on subsequent respiratory and pulmonary health.*** These data will provide a series of acute and longitudinal objective indicators of respiratory health and associated EC use, which is urgently needed to advocate for more effective regulations on ECs to reduce young adults use.

In summary, adolescence and young adulthood is a critical time for tobacco product experimentation, the escalation of tobacco use, and dependence. ECs are currently **the most used tobacco product among youth and young adults**, yet very little is known about what impact they may have on respiratory health. The proposed study will address this knowledge gap using a prospective, 1-year, longitudinal design combined with five intensive 2-week "measurement bursts" of EMA, spirometry, and nasal epithelial lining fluid (NELF) samples to assess respiratory health and inflammation. The longitudinal design will provide (a) an assessment of changes in patterns of EC and other tobacco use over time, and (b) the influence of daily EC quantity/frequency, type of EC liquid used, and dual use with OTPs impact on respiratory health among young adults.

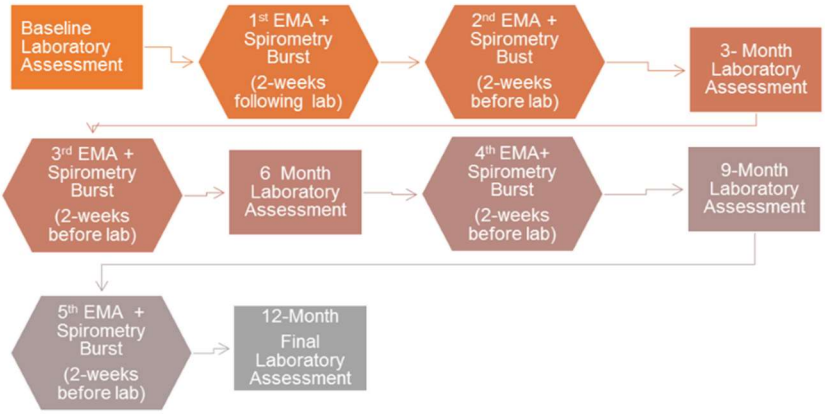
Preliminary Studies. Dr. Tackett completed a NIH F32 (F32HL138734) study, which incorporates secondary data analyses from the PATH study, qualitative interviews assessing why and how youth with asthma obtain and use ECs. Initial examination of study objectives indicate that youth with asthma are more likely to use ECs and OTPs than their healthy peers, which is consistent with the current literature.[9,10] While this study will provide preliminary evidence, from a relatively small sample ($N = 60$) regarding the acute health effects of, and reasoning behind youth EC use, it may only be generalizable to youth with asthma (8% of the US population of youth) and not to the larger population of youth who experiment with or use ECs (15-20% of the US population of youth). Further, youth with asthma already have impaired respiratory and pulmonary functioning, and thus our use of cross-sectional indicators of pulmonary health may be attributable to normative changes or nonadherence to asthma medications/poor disease management. By building on these initial findings to (1) provide a more complete assessment of EC use, (2) a more comprehensive, advanced, and longitudinal assessment of respiratory/pulmonary functioning, and (3) utilizing a "healthy" control group (i.e., referred to as "never users"), we can elucidate if changes in respiratory health are associated with EC use, rather than a confound of asthma status.

III. Procedures

A. Research Design.

Overview: Figure 1 (below) depicts the participant study flow. Using a 1-year, prospective longitudinal design, 150 young adults (100 exclusive-EC users; 50 never-users) will complete 5 study visits (baseline, 3-, 6-, 9-, and 12-months), which will include collection of nasal epithelial lining fluid (NELF) samples to examine objective respiratory outcomes and exposure/use of nicotine (i.e., cotinine). To assess acute changes in pulmonary functioning related to tobacco product use, participants will complete twice daily EMA and home-based spirometry for the 2-weeks prior to each follow-up study session visit. Exclusive-EC users' will provide self-reported data on frequency, quantity, and number of days ECs were used to examine the association between frequency and quantity of EC use and self-reported nicotine dependence and respiratory health.

Figure 1. Participant Study Visit Flow Chart



B. Sample

Participant Screening and Enrollment:
Given that EC use is more common during high school and young adulthood,[32-34,38,40,42,57,60,79,80] we will recruit a total of 100 exclusive-EC users and 50 never-users, ages 18-25. Participants will be recruited from the general community via targeted internet advertisements (i.e., Facebook, Instagram, and Twitter). Consistent with current protocols, interested participants will be screened over the telephone or in-person. Participants who meet the following eligibility criteria will be asked to take part in the study.

Inclusion criteria: 1) own a smartphone and willing to add study EMA and iSpirometry application to it; 2) a current exclusive-EC user (endorse ≥weekly use over the past 3 months) and not currently using other tobacco products; 3) between the ages of 18-25 years old at the time of enrollment; 4) willing to provide informed consent, 5) willing to complete five, 2-week periods of twice daily EMA and home-based spirometry, 5) read and speak English; and 6) **never-users** must indicate never trying any tobacco product to be eligible for enrollment.[11,82]

Exclusion criteria: 1) self-reported diagnosis of lung disease including cystic fibrosis or chronic obstructive pulmonary disease; we will not exclude youth who have asthma, but will incorporate this as a covariate during analyses; 2) unstable or significant psychiatric conditions (past and stable conditions will be allowed); 3) history of cardiac event or distress within the past 3 months; 4) are currently pregnant, planning to become pregnant, or breastfeeding (will be verified with urine pregnancy test); and 5) are blind, severely visually impaired, deaf, hard of hearing, or have a severe motor disability. If the participant is eligible then they will be invited to meet with the trained study RA to review study procedures and obtain informed consent. These procedures have been successfully implemented in previous protocols with our study team. *Upon completion of this study, we will provide participants with access to free tobacco dependence resources via the Tobacco Quit line (e.g.,* 1-800-QUIT-NOW*). If participants ask for smoking cessation resources or indicate they would like to quit, we will immediately provide these resources to participants (see protection of human subjects for more detailed information).*

Recruitment Feasibility and Retention. We will recruit our sample from a combination of resources, including online advertisements, and the databases of past participants. After a brief phone interview, eligible participants will join a video conferencing session (e.g., zoom, teams) where research staff will briefly explain the study, ensure inclusions/exclusion criteria, review and sign the informed consent, and enroll the participant.

Retention: Participants will receive the following compensation for taking part in this study: $25 for completing both the Baseline and Day 1 Visits, if the participant is eligible for the study ($25 total) $25 each for completing

the 3 Month, 6 Month, 9 Month, and 12 Month Visits (a maximum of $100) $5 for collect of NEFL sample at Day 1, 3 Month, 6 Month, 9 Month, and 12 Month Visits (a maximum of $25 total) $1 for completing each daily survey and $1 for completing each spirometry blow(a maximum of $280) $50 bonus for completing 75% or more (at least 105 total) of the daily surveys and attend at least 3 of the 5 remote study visits Participants who are eligible for the study can receive a maximum of $480 for participating. Participants who are determined ineligible during the consent process or at baseline for the study will receive $10 compensation for their time. We will also facilitate retention by: 1) obtaining multiple sources of contact; 2) offering evening/weekend appointments, and 3) provide reminder calls/texts/emails. The study app that will be added to each participant's phone will also send reminders of upcoming appointments and to complete EMAs.

C. Measurement / Instrumentation:

Questionnaire data will be collected via the MyCap app or over the phone by a research assistant using REDCap. See Table 2 for timing of measures. Pregnancy exclusion will be confirmed with a urine test. Exclusive-EC users and never-users will both complete objective measurements of respiratory health and self-report measurements of nicotine dependence.[51] *Spirometry* will be conducted in a standing position and according to the recommendations of ATS clinical Guidelines.[84] Forced expiratory volume (FEV1), forced vital capacity (FVC), and FEV1/FVC will be measured. *Nasal epithelial lining fluid (NELF)* samples will provide objective data on respiratory health (e.g., markers of inflammation, host defense, and lung injury) and will provide estimates of nicotine exposure/use via*cotinine* (i.e., the predominate metabolite of nicotine) will also be examined to provide objective indicators of recency of EC and/or OTP use. Participants will also complete a battery of self-reported questionnaires at each study session asking about demographic characteristics, nicotine dependence, frequency/quantity of EC use, perceptions of harm, and secondhand exposure to other tobacco products. *Drug Effects Questionnaire (DEQ)*.[45] A modified version of the DEQ will be used to rate EC appeal on visual analog scales (range, 0 "not at all" – 100 "extremely"). Items assess liking/wanting (the average of "I feel good EC effects," "I feel the EC strength," and "I like the EC effect"). *EC Wisconsin Inventory of Smoking Dependence Motives (e-WISDM)*.[78,79] The EC Wisconsin Inventory of Smoking Dependence Motives (e-WISDM) is a 37-item questionnaire that includes 11 subscales and two overarching subscales: Primary Dependence Motives (PDM) and Secondary Dependence Motives (SDM). *Minnesota Nicotine Withdrawal Scale (MNWS).*[79] Nicotine withdrawal will be assessed using the empirically validated 15-item version of the Minnesota Nicotine Withdrawal Scale. This measure assesses smoking craving, anger/irritability, anxiety, depressed mood, restlessness/difficulty concentrating, increased appetite, sleep problems, and somatic symptoms (nausea, constipation, sore throat, dizziness, coughing) rated on a scale of 0 (none/not at all) – 4 (severe/extremely).

Real-Time Assessment Procedures. Ecological Momentary Assessment (EMA): All participants will complete twice-daily EMA monitoring "bursts" for the 2-weeks prior to each scheduled laboratory visit (see Table 2 for specific EMA measures). Participants will receive prompts delivered to their phone in the morning and evening, corresponding to their sleep/wake cycle (assessed at baseline). Consistent with our team's previous studies, *EMA surveys* will assess current mood, nicotine craving, nicotine withdrawal symptoms, stress, recency of vaping/OTP use, current setting, frequency and quantity of EC and/or OTP use (since the previous assessment), flavors, nicotine concentration, EC device used, and satisfaction and pleasure from about their most recent EC use. The participants phone will audibly and visually cue EMA prompts lasting for 30 seconds. If the participant has not responded after 5 prompts (5 minutes apart), the assessment will be recorded as missed. Based on the experience Dr. Tackett's prior experience, EMA assessments take <5 minutes to complete. All EMA assessments will be date and time stamped for future analyses. Immediately after each EMA survey, participants will also complete handheld spirometry assessments (Figure 2, above). Participants will be prompted to complete spirometry assessments each day following completion of the EMA. Step-by-step directions to complete the spirometry test will be provided. The test is simple, easy to use, and provides a complete pulmonary function test in < 2 minutes. The EMA and iSpirometry applications will include a video to show participants how to complete the test, if additional support is needed. Results of these tests include peak flow and FEV1/FVC (Figure 2). All spirometry data are date and time stamped and saved within the application to a secure server. For the use of this study, participants will not receive feedback on spirometry test results.

TABLE 2. ASSESSMENT OVERVIEW AND OUTCOME MEASURES.

	Five Virtual "Laboratory" Sessions					EMA Measures				
BACKGROUND MEASURES	**Baseline**	**3-month**	**6-month**	**9-month**	**12-month**	Baseline	Month 2	Month 5	Month 8	Month 11
Sociodemographic Measures	•				•	• EC exclusive and OTP use - EC/OTP product used - Flavor used - Nicotine Concentration • Drug effects/liking • **Handheld Spirometry** • Affect/mood/stress • Minnesota Nicotine Withdrawal Scale • Hooked on Nicotine Checklist • Recency of product use • Social setting/location • Product experimentation (OTP and EC use)				
RESPIRATORY AND PULMONARY HEALTH MEASURES										
Spirometry	•	•	•	•	•					
NELF Biosample Collection (measures respiratory markers and cotinine)	•	•	•	•	•					
Urine pregnancy tests	•	•	•	•	•					
NICOTINE DEPENDENCE/ABUSE LIABILITY										
Drug Effects/Liking Questionnaire	•	•	•	•	•					
Minnesota Nicotine Withdrawal Scale (MNWS)	•	•	•	•	•					
Product Use and Dependence (Hooked on Nicotine Checklist; HONC)	•	•	•	•	•					
BIOMARKERS OF NICOTINE										
Cotinine	•	•	•	•	•					

D. Detailed Study Procedures

<u>Baseline Session:</u>

This is a completely remote, virtual study protocol and all sessions will be conducted via video conferencing software (e.g., zoom).

Prior to the start of the baseline session, the participant will be sent a personalized link to a video conferencing system (e.g., zoom). At the start of the baseline session, the participant must confirm that they are in a location where they can conduct the session, away from other people. The orientation session will occur. First, the study staff will confirm that the participant has a computer, laptop, tablet, or similar device with internet access that allows them to use video conferencing and complete REDCap surveys. The device must have a working camera, microphone, and audio. Second, the participant will answer questions to determine study eligibility and to confirm that the participant does not have COVID-19 via self-report. Third, study staff will review the informed consent and the informed consent must be signed by the participant prior to the administration of the baseline survey or the start of any study procedures. Fourth, the participant will read over the electronic informed consent form (ICF) and be given the opportunity to ask any questions that they may have. Prior to

signing the ICF, the participant will complete the consent quiz to assess their understanding of the study and their responsibilities. The study team member will then review any incorrect questions with the participant. After completing the consent quiz, the participant and study team member will electronically sign the informed consent form available in REDCap, if the participant agrees to participate in the study. A copy of the signed consent form will be emailed to the participant for their record.

For the baseline session, and visits for Day 1, 3-, 6-, 9-, 12-month sessions, participants will receive reminders through text, phone reminders, and/or Twilio/Apptoto. Upon completion of the consent, the study team member will then confirm the participant's address to receive all equipment and materials needed to conduct the study at home The participant will provide their availability to receive the study materials. If the participant is unavailable to receive the study package, another representative may receive the package instead. Participants will be asked to provide their social media contact information on a contact form. This information will only be used to contact participants if they change their address and/or phone number. We will not use this information to follow participants or collect information about them. Participants will receive a personalized link to the baseline study questionnaire, depending on if the person is a parent, adolescent, or young adult to complete while awaiting the receipt of the study materials.

Provision of Study Materials:

Between the baseline session and the first day of study participation, study products and other materials will be prepared and transported to the participant. Study materials will be prepared at our facility by staff. They will prepare a package with the requisite equipment and specimen collection materials to be picked up by the shipping/courier service. To ensure receipt of the package, the study team will track the location of the package and inform the participant of any updates. Materials will include: disposable protective gloves, alcohol wipes, pregnancy test (including a urine cup for collection to complete the test), a spirometer and nasal swab (NELF) kits. In addition, a package insert will be included inside the package to detail the materials/supplies that are included. With the arrival of the study materials, the study team will contact the participant to schedule the first of five (5) EMA 2-week assessment monitoring bursts.

Study Session - Day 1

Once the participant has completed the consent and baseline questionnaires, and received the study materials, they will attend the Day 1 study session. This session will last up to 30 minutes. At the start of the session, the participant will be instructed to put on the enclosed gloves and wipe down the spirometer and one nasal swab (NELF) collection kit. The study team member will instruct the participant on how to complete the twice-daily ecological momentary assessments (EMA) questionnaires during each 2-week EMA study period through the secure smartphone application, RealLife Exp, by LifeData LLC. Participants will be provided instructions on how to download the application on their smartphone, how to download the relevant study survey package, and how to enter their study ID. This application will push survey notifications to participants' phones in the morning and evening each day corresponding to their sleep/wake cycle (assessed at baseline). If participants does not complete the EMA survey immediately, RealLife Exp will send a reminder notification every 30 minutes to encourage survey completion. Prompts not completed within 90 minutes (5 reminders total) will disappear and be marked as missed. EMA surveys will assess current mood, nicotine craving, nicotine withdrawal symptoms, stress, recency of vaping/OTP use, current setting, frequency and quantity of EC and/or OTP use (since the previous assessment), flavors, nicotine concentration, and EC device used. EMA assessments will take less than 5 minutes to complete. All EMA assessments will be date and time stamped for future analyses.

The study team member will then show the participant how to use the spirometer using an instruction video. Participants will be prompted to complete twice daily spirometry assessments following completion of the daytime and nighttime EMA surveys. Step-by-step directions to complete the spirometry test will be provided. The test provides a complete pulmonary function test in less than 2 minutes using the Spirobank application.

Results of spirometry tests include peak flow and FEV1/FVC. All spirometry data are date and time stamped and saved within the application to a secure server. The study team member will instruct the participant on how to collect the nasal swab specimens and review videos that demonstrate the appropriate collection techniques. At each virtual session (3-, 6-,9-,12-month visits), participants will also complete nasal swab collection. Step-by-step directions to complete the collection and return it to the lab will be provided with the study team member facilitating picking up the specimen. Participants will be instructed to place specimens and the freezer pack in their freezer (for no more than one week) until the pick-up. Prior to the end of the session, the staff will request that the participant either take a picture of their e-cigarette device and e-liquid or show the study team member their e-cigarette device and e-liquid. The information will allow the study team to code for e-cigarette device specifications (i.e., voltage, disposable e-cigarette device, etc) to better understand participant preferences.

Never/non-users will also complete up to a 30 minute individual interview with a study team member who will ask about reasons for non-use. To protect the anonymity of the participants, staff will instruct the participant turn off their camera and complete the interview with only their participant ID on the zoom screen. We never conduct the video or record the session with their camera on to protect their anonymity. Upon the verbatim transcription of the session, we will completely delete the zoom video recording. In addition, we only begin recording when the participant has turned off their camera and end the recording before their camera to turned on again. All videos will be deleted at the end of the study and only the verbatim transcripts will be used for qualitative data analyses. After the interview, study staff will complete a debrief form to assess interview saturation. This is helpful to determine when/if all topics related to non-use have been discussed. The audio of the interview will be recorded and transcribed (both will be saved on a secure server and coded with the participant's study identifier).

EMA Study Session - 2 week following Day 1, then 2-weeks prior to each virtual visit (3-, 6-, 9-, 12-month sessions)

The participant will continue inputting EMA surveys and utilizing the spirometer for a 2-week period. All participants will complete twice-daily Ecological Momentary Assessment (EMA) monitoring “bursts” for the 2-weeks prior to each scheduled zoom visit through RealLife Exp, a secure smartphone application by LifeData LLC. The LifeData platform (www.lifedatacorp.com) will be used to develop the application and securely collect data. The participants will be instructed to download the LifeData application on their smartphone and to download the relevant study survey package to start participation. Prompts that are not completed within 90 min of the notification alert will disappear and will be marked as missed. Consistent with our team’s previous studies, EMA surveys will assess current mood, nicotine craving, nicotine withdrawal symptoms, stress, recency of vaping/OTP use, current setting, frequency and quantity of EC and/or OTP use (since the previous assessment), flavors, nicotine concentration, EC device used, and satisfaction and pleasure from about their most recent EC use. Based on Dr. Tackett’s prior experience, EMA assessments take <5 minutes to complete. All EMA assessments will be date- and time-stamped for future analyses. Participants will be prompted to complete twice daily spirometry assessments following completion of the daytime and nighttime EMA surveys. Step-by-step directions to complete the spirometry test will be provided. The test is simple, easy to use, and provides a complete pulmonary function test in < 2 minutes. All spirometry data are date and time stamped and saved within the application to a secure server. At the end of the 12 Month visit, their participation in the study is complete.

3-, 6-, 9-, 12-Month Sessions

Prior to each session, EC-users will complete questionnaires similar to the baseline assessment (e.g., EC use, OTP use, demographics, respiratory health, etc.). Participants are asked to complete these questionnaires before the start of the session. At the session, the study staff will check in on how the study is going, use of the spirometer/EMA applications and answer any questions the participants may have. Each session will last about

1 hour. Prior to each session, never/non users will complete questionnaires similar to the baseline assessment (e.g., any EC use, OTP use, demographics, respiratory health, etc). Participants are asked to complete these questionnaires before the start of the session. At the session, the study stuff will check in on how the study is going, use of the spirometer/EMA applications and answer any questions the participants may have. Never/non-users will also complete up to a 30-minute individual interview with a study team member who will ask about reasons for non-use. Study staff will complete the debrief form to assess interview saturation. This is helpful to determine when/if all topics related to non-use have been discussed. Each session will last up to 1 hour. The study team member will instruct the participant on how to collect the nasal swab specimens and review videos that demonstrate the appropriate collection techniques. At each virtual session (3-, 6-,9-,12-month visits), participants will also complete nasal swab collection. Step-by-step directions to complete the both collections and return them to the lab will be provided with the study team member facilitating picking up the specimen. Participants will be instructed to place specimens and the freezer pack in their freezer (for no more than one week) until the pick-up.

Final Visit 12-month session

Participants will complete their final session by answering questionnaires similar to the baseline assessment (e.g., any EC use, OTP use, demographics, respiratory health, etc.), final nasal sample, and a questionnaire asking about their self-reported rating of how the study went and any thoughts about their responses. When participants return their final nasal sample, they will also be asked to return their spirometry device.**Protection Against Risk:**

The research protocol calls for current exclusive- EC and never-users (never tobacco users). Those who participate in this study will be asked questions about EC use and other tobacco product (OTP) use. Currently ECs are the most commonly used tobacco product among adolescents and young adults. We will never provide any tobacco products to participants, encourage participants to begin using ECs or OTPs, and will provide smoking cessation to those interested or ask about referrals at each visit. Questionnaires, respiratory assessments, and NELF collection procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) risk of using ECs, b) dual use of cigarettes and ECs, c) loss of confidentiality or privacy, and d) potential for undermining smoking cessation.

F.2.B. Adequacy of Protection against Risks

Recruitment and Informed Consent: At first contact, all participants 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 oral 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 script and materials, consent forms and all study procedures will be approved by the OSU Institutional Review Board. All participants will provide consent before any study data is collected.

F.2.C. Protections Against Risk:

Risk of using ECs: The risk of side effects and adverse events are very low. These products are sold online, and at EC specialty stores and convenience stores nationwide, without a prescription. Nevertheless, all participants will be screened for general medical precautions (e.g., 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. Any serious adverse events will be reported to the study's DSMB and then to the OUHSC IRB and 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 our team's previous studies) and mild (nausea, headache, disrupted sleep), and will be handled quickly (i.e., advice to participant to reduce or stop EC use). Lab studies of toxin exposure suggest that ECs incur no greater risk to health than do conventional cigarettes. Indeed, ECs generally show lower levels of

harmful and potentially harmful constituents. To date, five EC 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 EC group and the nicotine patch group had comparable levels of adverse events. The most common were mouth irritation, throat irritation, dry cough and headache. Further, participants will be using their own EC device and we will never provide tobacco products to young adults at any time.

Risk of dual use of cigarettes and ECs: The concern of EC users 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 trials with ECs, none of the participants reported any indication of nicotine overdose in their dual use of ECs and OTP. The literature indicates[33,38,40,79,80] that, at most, 20% of the participants enrolled in the proposed study will become a dual user over the 1-year period (estimated *n* = 30). We will provide free smoking cessation referrals for young adults and provide informational resources regarding the risks of dual using ECs with combustible OTPs (see Potential for Undermining Cessation below).

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 in separate from participant data. Only study research assistants and the PI will have the information that connects participant's name and ID number. All electronic data will be numerically coded and stored in a password protected database, on a password protected computer in a secure research space.

EMA Confidentiality Procedures. The following features are designed to address smartphone/EMA data security issues: 1) the data stored on the smartphone device is in a SQLite database in a sandbox environment where read/write operations are only available through the programming application. No file or output is readable to end users, 2) a password (only known to researchers) is required to authenticate the current user before data can be downloaded from the smartphone device to the server, 3) the web browser application linking the principal investigator's computer to the database is on HTTPS protocol (SSL certificate with encryption) which will guarantee the data transfer from web browser to the backend database is well protected. Data will be downloaded frequently and saved on OSU approved servers (e.g., T-drive at the Center for Tobacco Research) which meets OSU security requirements. . Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will never be reported in any publication.

Potential for Undermining Cessation: The study sample is comprised of current exclusive-EC and never-users. We will never ask users who are in the process of quitting to stop. At the end of the study, all participants will be debriefed and educated about ECs and OTP. 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 largely unregulated by the FDA until they are able to develop appropriate guidelines, and e) it is unclear whether ECs reduce the risks associated with smoking. The PI and Primary Mentor 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 will be no greater harm, as the participants in the "user" group have already decided on his/her own to use the product. While some young adults in the present study will be under the legal age of tobacco use, ECs are available online and over-the-counter at various convenience stores, EC specialty stores and places where tobacco products are sold. Further, we will never provide any product to young adults. 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 participants will be told that if at any time during the study they are interested in smoking cessation services, a list of free smoking cessation resources will be provided, including the Quit line.

Adverse Events:

All observed or volunteered adverse events regardless of tobacco use status (exclusive EC-user or never-user) will be recorded on the adverse event page(s) of the Adverse Event Form. Exacerbation of pre-existing illness is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the study. It may include worsening or increase in severity of signs or symptoms of the illness, increase in frequency of signs and symptoms of an intermittent illness, or the appearance of a new manifestation/complication. Exacerbation of a pre-existing illness should be considered when a patient/subject requires new or additional concomitant medication or non-medication therapy for the treatment of that illness during the study. Lack of or insufficient clinical response, benefit, efficacy, therapeutic effect, or pharmacologic action should not be recorded as an adverse event.

For all adverse events, Drs. Tackett and Wagener will pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to OSU IRB/ NIH. For all adverse events, sufficient information should be obtained by Drs. Tackett and Leventhal to determine the causality of the adverse event (i.e., other illness). Drs. Tackett and Wagener is required to assess causality and indicate that assessment on the Case Report Form. Follow-up of the adverse event, after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator or his/her designated representative.

All serious adverse events will be reported immediately to Dr. Brown then to IRB and the NIH. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient/subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Any serious adverse event or death must be reported immediately independent of the circumstances or suspected cause if it occurs or comes to the attention of the principal investigator at any time during the study through the last follow-up visit required by the protocol. Any serious adverse event occurring at any other time after completion of the study must be promptly reported. For all serious adverse events, the investigator is obligated to pursue and provide information as requested by the DSMB in addition to that on the Adverse Event Form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including concomitant medications and illnesses, must be provided. The investigator's assessment of causality must also be provided. Drs. Tackett and Wagener will ensure that information reported immediately by telephone or other means and information entered in the Adverse Event Form are accurate and consistent.

We will monitor for risk of using ECs by screening participants for general medical precautions. The most likely adverse event (potential for nicotine overdose) is anticipated to be rare and mild based on Dr. Wagener's previous studies, and will be handled quickly (i.e., advice to participant to reduce or eliminate nicotine use). Lab studies of toxin exposure suggest that EC incur no greater risk to health than do combustible cigarettes. Dr. Tackett and study personnel will be available for any questions that participants may have about ECs, smoking, or smoking cessation. Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and Drs. Tackett and Wagener.

E. Internal Validity

Quality assurance: Procedures in place to ensure the validity and integrity of the data. It will be made clear to participants that all information obtained during assessments is confidential and that no information will be shared with the participants' clinicians unless the participant requests this in writing. All investigators and staff associated with this project have been trained, and new hires will be trained on human research ethics in accordance with the requirements of the local institutions during initial project approval.

Drs. Tackett and Wagener will train and closely monitor the Research Assistants on the procedures to be

used in this study. Such monitoring will consist of frequent in-person discussion of study visits at the beginning of the study and less frequent monitoring as the study progresses.

Participants will be trained during the Day 1 Session on how to operate the study app, and participants will have a chance to ask any questions during the subsequent calls. The app will remind participants about when to complete assessments.

Data will be collected in a consistent manner across all years of the study. Standard operating procedures will be developed.

Data collection/entry/transmission/analysis: Many subjective measures will be administered using our study app, MyCap. MyCap syncs with RedCap, and thus, all data are entered directly into a RedCap database via the study app. These will be identified by Subject Identifier. Forms will include programming features to ensure valid data (i.e., input masks, validation criteria, skip logic) and will be stored at the Ohio State University.

F. Data Analyses

Power Analyses. For Aims 1 and 2: The use of repeated measures and longitudinal methodology allow for greater detection of moderate effect sizes associated with decreased in markers of respiratory health, increases in nicotine addiction, changes in EC and/or OTP use patterns, and frequency/quantity of EC use. For **each** repeated measures analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) we are 95% powered to find moderate effect sizes. For Aim 3: Power was calculated using MPlus Monte Carlo estimation informed by published and preliminary studies. To test power for MLM, we first examined power with Optimal Design using repeated measures. We then repeated our power analyses in MPlus Monte Carlo estimation, building on published MLM simulations and using 1000 replications to confirm stability of each model, to better capture the complexity of the separate models, including cross-level interactions. For the EMA analyses, the power of the statistical test depends on the total effective sample size (ESS) and the statistical model used. The total ESS is the number of statistically independent observations available for this study. The number of statistically independent observations is the total number of observations (number of participants x number of data collections) adjusted for within-individual correlations. As the within-individual, or intra-class, correlation (ICC) increases, the ESS decreases. The following formula illustrates this relationship, whereby the effective sample size is equal to $nm/(1+(m-1)\rho)$, where n = number of participants; m = number of repeated measures for each participant; and ρ = ICC.[94] In sum, even assuming effects as small as .10 (well below expected effect), we will be well powered (> .85) to identify effects across analyses.

Data Analytic Overview. Analyses will be conducted using SAS, R and MPlus. For Aims 1 and 2, distribution plots will be carried out on all data to assess for normality, and any non-Gaussian data were evaluated with either Wilcoxon signed rank test or, where appropriate, data will be logarithmically transformed before analysis. Comparisons of continuous data will be made by an overall analysis of variance, followed by Bonferroni corrected, multiple-range testing, to obviate multiple pairwise comparisons, with the overall α error set at .05 (2tailed). For Aim 3, as appropriate, alternate estimators to address expected and observed characteristics (e.g., Poisson distribution, zero-inflation) will be used. MPlus permits frequentist and Bayesian estimation for models with missing data. Bayesian procedures allow multiple imputation of multiple datasets subsequently used with parameter estimates averaged across imputed datasets. Multiple imputation will virtually always outperform older methods of managing missing data (e.g., listwise deletion) with regard to both accuracy and power, making it the preferred approach.[72, 73] Measurement models will be formally tested. If analyses do not support the presence of the expected unitary constructs, subsequent analyses will examine aims as defined by observed variables. Model invariance tests will also be used to assess appropriate statistical treatment of gender; if measurement invariance is not observed, analyses will be stratified by gender. Aim 1: Hypotheses 1a and 1b: We will use two-way repeated measures ANOVA and MANOVA with Tukey adjustment to examine differences between the measures of respiratory health (pulmonary functioning, NELF samples) and EMA data (five two-week monitoring periods). For *H1a*, two-way repeated measures ANOVA will compare mean differences between pulmonary and

respiratory markers and user status (EC-exclusive vs. never-users). For ***H1b***, we will use MANOVA models to examine changes in frequency/quantity of EC use and nicotine dependence (significant increases in HONC scores) across each session with respiratory and pulmonary functioning. Aim 2 Hypotheses 2a and 2b: We will use two-way repeated measures analysis of variance and multivariate analysis of variance with Tukey-adjustment to examine differences between the number of vape sessions per day and time spent vaping with pulmonary functioning across each EMA monitoring period. For ***H2a***, two-way repeated measures ANOVA will compare mean differences between pulmonary and frequency/quantity of EC use. For ***H2b***, we use MANOVA models to examine changes in pulmonary functioning and the experimentation of OTP in conjunction with EC-use across the 1-year monitoring period. Exploratory Aim 3: Hypotheses H3a: We will use multilevel models (MLM) to examine the daily-level associations between the frequency/quantity of EC use experimentation and pulmonary functioning. These models will allow us to examine the within-person associations between EC use and pulmonary functioning, using Curran and Baurer's[95] procedures for disaggregating within-person and between-person effects using two level analysis in multilevel modeling. These models will also allow for the examination of cross-level interactions among between and within-person factors. Specifically, we will be able to examine the role of daily within-person fluctuations in frequency/quantity of EC use in its links to pulmonary functioning. Further, we will be able to assess the potential differences by user status as well as baseline respiratory health. Significant interactions will be probed using Preacher, Curran, & Bauer's[96] computational tools. The applicant has integrated training in these techniques into her goals (Goal #4).

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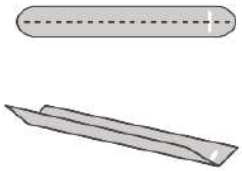
V. Appendix A. Nasal epithelial lining fluid (NELF) Sample Procedure Schematic

1. Prepare Saline and Prime Spray Bottle

2. Spritz Each Nostril with Saline

3. Bend NELF Strip in Half "Hotdog Style"



4. Insert NELF Strip to Each Side of Nose Until Slit Reaches Edge of Nostril

5. Clamp Nose for 2 Minutes

6. Remove NELF Strip From Each Side of Nose

7. Transfer NELF Strips to Labeled Microtubes and Store in Freezer