Behavioral Activation for Smoking Cessation and the Prevention of Post-Cessation Weight Gain

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted in compliance with this research protocol, as well as according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and institutional research policies and procedures.

1.1 Background - Parent (Main) Study

Smoking cessation and the prevention of obesity are two of the most significant cancer prevention outcomes. Smokers who want to quit smoking and minimize weight gain have few, if any treatment options, despite two decades of post-cessation weight gain (PCWG) research. Identifying effective PCWG prevention approaches has the potential to remove a major barrier to the initiation and maintenance of smoking cessation, and to reduce the overweight and obesity problem in the US. To advance the science and practice of interventions to reduce PCWG, we propose to evaluate a novel application of a behavioral activation (BA) intervention to prevent PCWG in men and women. To date, PCWG prevention research has focused on women, even though 25% of the increase in the proportion of overweight men has been attributed to quitting smoking. If the hypotheses are supported, the findings would suggest that targeting reward-related mechanisms common to both smoking and eating: (1) is an effective approach to promoting smoking cessation, while minimizing PCWG; (2) is a viable approach for addressing two rewarding behaviors concurrently, without risking compensatory responses; and (3) can be used as an adjunct to pharmacotherapy. Finally, the findings have implications for improved treatments for multiple co-morbid health risk behaviors (obesity, alcohol, substance use), supporting a wide-ranging impact on the prevention of cancer and other diseases.

Behavioral theories, such as Behavioral Economic and Incentive Salience Theories suggest that PCWG may stem, in part, from the reward deficit produced by smoking cessation. Upon quitting, smokers lose a significant reinforcer, increasing the motivational salience of available alternative reinforcers. However, smokers typically have fewer alternative reinforcers from which to choose and may experience less pleasure from these reinforcers after quitting smoking. Compensatory increases in between-meal snacking on foods high in fat and sugar may offset the reward deficit due to quitting smoking. Highly palatable snack food is a readily available reinforcer that shares common reward mechanisms with nicotine. Behavioral Economic Theory indicates that the reinforcing value of snacking can be enhanced, or reduced, based on the availability of alternative reinforcers. Fewer alternative reinforcers and a reduction in pleasure derived from available reinforcers forge an over-reliance on palatable snack foods to substitute for the reinforcement previously derived from cigarettes. After quitting smoking, individuals may become more responsive to snack food cues, find snack foods more pleasant to eat and more reinforcing. Indeed, our research group has shown that smoking cessation increases the reinforcing value of snack foods, which predicts subsequent food intake and weight gain. To avoid smoking cessation-induced increases in food intake and weight gain that precipitate smoking relapse, it is key to evaluate novel behavioral interventions that target involvement in, and subjective reward derived from, alternative reinforcers.

To advance the science and practice of interventions to reduce PCWG, we propose to target reward-related mechanisms common to smoking and palatable food intake. Through a novel application of a behavioral activation intervention to smoking cessation and to PCWG (BAS+), we will increase opportunities for reinforcement and enhance the pleasure obtained from typical reinforcers. We propose a randomized clinical trial of BAS+ plus transdermal nicotine (TN) vs. Standard Smoking Cessation Counseling (SC) plus TN in treatment seeking smokers (ages 18-65). Participants will receive 8 individual BAS+ or SC sessions plus TN over a 10-week period.

The primary outcomes will be PCWG and 7-day point prevalence abstinence at 26 weeks after the target quit date. Food intake (secondary outcome) will be measured via 24-hour food recalls conducted over 3 consecutive days at pre-treatment, mid-treatment, end of treatment, and follow-up. Mediating mechanisms (e.g., alternative reinforcers, relative reinforcing value of food, food reward, and food cue-induced craving) will be assessed before, during, and at the end of treatment (week 8). Potential moderators include gender, depression symptoms and weight concerns.

1.2 Background - Neuroimaging Sub-Study

The parent randomized clinical trial of behavioral activation intervention (BAS+) integrates Behavioral Economic Theory and cognitive neuroscience research. The basic premise is that smoking cessation produces a reward dysregulation that increases the reward value of other reinforcers, such as highly palatable foods. Because food is a readily available reinforcer --and palatable foods increase in their incentive salience after quitting-- food cue-elicited reactivity in the brain's reward system would increase with smoking cessation. Food reward and reinforcing value also increases in the context of cessation leading to increased food intake. Cessation-induced increases in food reward are paralleled by decreases in the ability to control the consumption of palatable food. Research by our group and others shows that cessation impairs aspects of executive function that are critical to self-control. Abstinence reduces engagement of brain regions essential for cognitive control and individuals with lower levels of response inhibition are less able to resist palatable foods, and are more vulnerable to overeating. Working memory is important to keep goals in mind and for efficient use of behavior change tools, yet it can be depleted by efforts to manage cravings. This will be the first study to integrate these concepts and to explore brain mechanisms underlying a behavioral activation intervention (BAS+) to promote smoking cessation and mitigate PCWG.

This neuroimaging study will aim to examine a sub-set of the randomized clinical trial participants. Functional magnetic resonance imaging (fMRI) can identify mechanisms underlying behavior change beyond self-report and behavioral measures. We will examine neural responses in three parallel pathways including: cognitive control, food cue reactivity, and food reinforcement. As in the parent study, the primary outcomes will be food intake and 7-day point prevalence abstinence at 12 weeks post target quit date. BOLD signal (secondary outcome) will be measured at pre-treatment (week -2) and end of treatment (week 8). Our three aims address: 1) identifying putative brain mechanisms underlying BAS+ (vs. SC) treatment for smoking cessation and PCWG; 2) evaluating the relative contribution of treatment-induced changes in task related brain signal (Food Cue Response, Food Reinforcement/Choice) in prediction of post treatment food intake; and 3) testing whether BAS+ (vs. SC) induced change in neural responses to food cues and food reinforcement is a stronger predictor of food intake among smokers with greater cognitive control. The findings will address *why* BAS+ may be effective in managing PCWG and provide insight into which patients are best supported by this treatment. We also test whether cognitive control processes moderate the influence of BAS+ treatment on food intake.

1.3 Background- Microbiome Sub-Study

The Gut Microbiome May Underpin the Substitution of Food for Cigarettes After Smoking Cessation: Converging research suggests that the gut microbiome may play an important role in modulating post-cessation increases in food intake and set the stage for smoking relapse. Gut bacteria and the brain have a bi-directional route of communication through the microbiome-gut-brain (MGB) axis. As such, smoking cessation-induced perturbations in the gut microbiome have the potential to impact the brain as well as behavior. Preliminary research has shown that smoking cessation results in profound changes in gut microbial abundance within 4

to 8 weeks after quitting smoking (see Aim 1). These smoking cessation-associated changes (e.g., reduced *Bacteroides,* increased *Firmicutes*) are similar to those observed in obesity.

Smoking cessation-induced changes in the abundance of gut bacteria likely have functional, downstream consequences for dietary intake and smoking abstinence. Gut microbes secrete neurotransmitters and other neuroactive metabolites that regulate reward-seeking behavior by communicating pleasure and satiety to the brain via the vagus nerve. For example, dopamine, serotonin, and gamma-aminobutyric acid (GABA), as well as their precursors (tyrosine, tryptophan, and glutamate, respectively), are secreted by multiple genus. Indeed, the gut is a significant source of these key neurotransmitters that are also active in the brain. The gut microbiome also produces neuroactive metabolites (e.g., indole and short chain fatty acid propionate) that impact appetite by stimulating the production of gut hormones with anorectic functions. Shifts in the abundance of bacteria that produce these neurotransmitters and metabolites likely translate to a reduction in these molecules that are involved in reward and appetite regulation. In turn, reduced reward and appetite regulation may drive the substitution of cigarettes with palatable snack foods, as well as smoking relapse.

Can BAS+ Mitigate the Effects of Gut Microbiome Changes on Dietary Intake and Smoking Relapse? Smoking cessation induced changes in the composition of the gut microbiome along with downstream reductions in neuroactive metabolites involved in reward regulation and appetite control likely result in increased food intake (and ultimately weight gain) and smoking relapse. As such, the gut microbiome may provide a biological basis for the observation that smoking cessation heightens the reinforcing value of snack foods, which predicts subsequent food intake and weight gain. In the context of fewer alternative reinforcers and diminished sensitivity to these reinforcers, there is little to compete with food or cigarettes as a reinforcer. BAS+ focuses on increasing the number of, and pleasure derived from, alternative reinforcers to improve cessation rates, mitigate increases in dietary intake, and lesson PCWG. We will explore whether BAS+ (versus SC) mitigates the effects of gut microbiome changes on dietary intake and smoking abstinence at 12 weeks post TQD.

2 Study Objectives

2.1 Parent (Main) Study

Aim 1: To evaluate the efficacy of BAS+ versus SC when delivered in conjunction with TN.

H1a: Compared to SC, participants in the BAS+ group will have less post-cessation food intake and gain less weight at week 26.

H1b: Compared to SC, participants in the BAS+ group will have higher smoking cessation rates at the end of treatment and at the 12- and 26-week follow-up time points.

Aim 2: To examine the mechanisms by which BAS+ reduces PCWG and promotes smoking cessation.

H2a: BAS+ vs. SC will increase engagement in and enjoyment from alternative reinforcers, and reduce the relative reinforcing value of food, food reward, and food cue-induced craving after smoking cessation.

H2b: Changes in these measures will predict reduced food intake and weight gain, which in turn will predict increased quit rates.

Exploratory Aim: We will explore whether women, or smokers with greater pre-treatment weight concerns or depression symptoms are more likely to exhibit PCWG and more likely to benefit from BAS+ vs. SC.

The proposed study breaks new conceptual and empirical ground by: (1) providing the first evaluation of the efficacy of BAS+ for smoking cessation and PCWG as an adjunct to the most widely used pharmacotherapy for smoking cessation and (2) examining the mechanisms by which BAS+ reduces food intake and weight gain. If the hypotheses are supported, the findings would suggest that targeting reward-related mechanisms common to both smoking and eating: (1) is an effective approach to promoting smoking cessation, while minimizing PCWG; and (2) is a viable approach for addressing two rewarding behaviors concurrently, without risking compensatory increases in one of those behaviors. Pretreatment assessments will enable the identification of potential individual differences in intervention efficacy.

2.2 Neuroimaging Sub-Study

Aim 1: Identify putative brain mechanisms underlying BAS+ (vs. SC) treatment for smoking cessation and PCWG.

H1a: BAS+ (vs. SC) will decrease food cue-induced activity in insula, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and ventral striatum/accumbens (VS/NAC);

H1b and H1c: (Hb) BAS+ (vs. SC) will decrease food reinforcement choice-related activity in VS/NAC and ventromedial prefrontal cortex (vmPFC); and **(H1c)** will produce corresponding decreases in food craving, reward, and reinforcement at the behavioral level (task performance).

Aim 2: To evaluate the relative contribution of treatment-induced changes in task related brain signal (Food Cue Response, Food Reinforcement/Choice) in prediction of post treatment food intake.

H2a: Brain network response (H1a-b) in the BAS+ (vs. SC) group will characterize treatment-induced changes in caloric intake (and quit success) more accurately than clinical (age, sex, nicotine dependence, BMI) and behavioral (withdrawal, craving, food reinforcement) measures alone.

H2b: The relative contribution of domain specific brain signal (food cue reactivity and food reward) to the prediction of treatment-induced change in caloric intake will be evaluated (regression model).

Aim 3: Test whether BAS+ (vs. SC) induced change in neural responses to food cues and food reinforcement is a stronger predictor of food intake among smokers with greater cognitive control.

We predict that the relationship of food cue and choice-related neural activity (e.g., VS/NAC BOLD signal; BAS+ vs. SC) will be a stronger predictor of caloric intake among smokers who also show greater working memory-related neural activity (i.e., DLPFC BOLD signal) at baseline and post treatment. Regions of interest for food cue and choice-related neural activity will be selected from those shown to be sensitive to cessation in Aim 1.

Utilizing fMRI, we can identify the neural underpinning of a novel behavioral activation (BAS+) intervention for smoking and related PCWG. The findings will address *why* BAS+ may be effective in managing PCWG and provide insight into which patients are best supported by this treatment. We also test whether cognitive control processes moderate the influence of BAS+ treatment on food intake. If our cognitive control hypothesis is supported, combining neurocognitive training with BAS+ could improve treatment efficacy. The integration of concepts and tools from behavioral economics and cognitive neuroscience tackles PCWG in a highly novel way, with implications for validating new treatments.

2.3 Microbiome Sub-Study

Aim 1: To identify earlier (4-weeks post TQD) and persistent (8-weeks post TQD) smoking cessation associated changes in gut microbiome composition and function.

H1a: Smoking cessation will result in decreases in *Prevotella* and *Bacteroides* species from the *Bacteroidetes* phylum, decreases in β- and ɣ- *Proteobacteria*, increases in *Clostridium* clusters IV and XIV from the *Firmicutes* phylum, and increases in *Bifidobacteria* and HGC bacteria from the *Actinobacteria* phylum.

H1b. We expect to observe decreases in neurotransmitters implicated in reward (dopamine, serotonin, GABA) as well as their precursors (tyrosine, tryptophan, glutamate), and microbial metabolites involved in appetite regulation (indole and propionate).

Aim 2: To evaluate the relationship between post-cessation changes in the gut microbiome, dietary intake, and smoking abstinence.

H2: Greater post-cessation changes in the gut microbiome will predict increased dietary intake and lower smoking abstinence rates 12 weeks post TQD.

Aim 3: To explore whether BAS+ (vs. SC) mitigates the effect of gut microbiome changes on dietary intake and smoking abstinence.

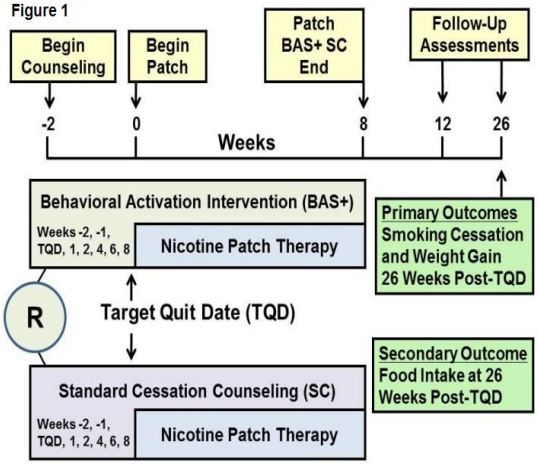
H3: Because BAS+ promotes alternative, non-food sources of reinforcement, BAS+ (vs SC) will mitigate the effects of microbiome changes on dietary intake and smoking abstinence 12 weeks post TQD.

3 Study Design

3.1 General Design - Parent (Main) Study

This is a randomized clinical trial of the efficacy of BAS+ plus TN compared to SC plus TN in treatment-seeking smokers. After completing an Intake Visit (~week -3), eligible smokers will be randomized (stratified by nicotine dependence, BMI and gender) to participate in 8 individual sessions of BAS+ or SC over a 10-week treatment period with two sessions prior to the target quit date (weeks -2, -1) and six sessions post-target quit date (TQD [week 0] and weeks 1, 2, 4, 6, and 8). Standard, 8-week; open-label TN will begin on the TQD. Moderators will be assessed pre-treatment. Mediating mechanisms will be assessed before, during, and at the end of treatment (EOT, week 8). Smoking will be assessed by self-report and biochemically confirmed (Carbon Monoxide [CO] < 5 or absence of Urine Cotinine) at all in-center visits (for CO ONLY) after quitting, at EOT (week 8), and at the 12- and 26-week follow-ups. Weight will be assessed at these same time points. Food intake will be measured by three consecutive days of 24-hour food recalls at Baseline [week -2] and 4-, 8-, and 12- and 26-weeks post-TQD. Smoking cessation and PCWG are the primary outcomes and food intake is a secondary outcome at 26-weeks post-TQD. Consistent with intent-to-treat (ITT) analyses, we will measure smoking cessation and weight gain in the full sample at the 26-week follow-up, evaluating a smoking status by treatment interaction for the PCWG analysis.

Figure 1



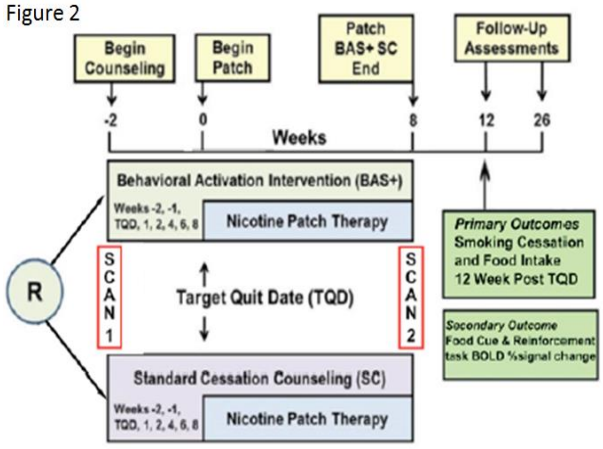
Time points (i.e. study weeks) displayed in **Figure**

1 and included within the Study Procedures Section represent approximations. Participants will follow a study schedule similar to that designated in Figure 1 and the Study Procedures, but will be permitted a degree of flexibility based on the maximum/minimum amount of time allowed between each time point established by the Principal Investigator. If a participant is unable to attend an in-person visit within the permissible window of time allotted between time points, counseling and applicable study measures may be completed/collected via telephone. In-person completion of the Intake and Baseline Visits are considered mandatory for study participation.

3.2 General Design - Neuroimaging Sub-Study

We propose to study a sub-sample of consecutively recruited participants (N=~50) enrolled in the parent randomized clinical trial. As displayed in **Figure 2**, an equal number of fMRI eligible smokers from the BAS+ or SC treatment group will be studied before treatment (~week -2) and during the final week of nicotine patch therapy (~week 7-8). As part of the parent study, smoking will be assessed by self-report and biochemically confirmed weekly after quitting, at EOT (week 8), and at the 12 and 26 week follow-ups. Weight will be assessed at these same time points. Food intake will be measured by three consecutive days of 24-hour food recalls at baseline and 4-, 8- 12- and 26-weeks post-TQD.

Figure 2



Scans identified in **Figure 2** and included within the Study Procedures Section represent approximations. Participants will complete scans as designated in Figure 2 and the Study Procedures, but will be permitted a degree of flexibility at the discretion of the Principal Investigator due to extenuating circumstances.

3.3 General Design - Microbiome Sub-Study

We will study a subsample of up to 60 consecutively recruited smokers enrolled in the parent trial. The microbiome study subgroups will be balanced for age, sex, and BMI. In addition to the exclusion criteria for the parent trial, we will exclude individuals with current and recent (last 3 weeks) use of non-topical antibiotics, antifungals, antivirals, and psychotropic drugs. Participants who are initially eligible based on telephone screening will complete an in- person Intake session as part of the parent study. Before or during the Intake session, consent for participation in the sub-study will be obtained and final sub-study eligibility will be confirmed. An equal number of microbiome eligible smokers from the BAS+ or SC treatment groups will be instructed to provide a stool sample on **Week -2 (Baseline Visit), Week 4 (Mid-Tx 3 Visit)**, and **Week 8 (End of Treatment Visit after completing TN treatment)**. Participants will be instructed to collect a stool sample within 24 hours (up to ~36 hours is permissible) of the target time points. Directions and supplies for stool specimen collection will be provided at the visit prior to collection.

We will assess within-subjects changes in the gut microbiome across time and explore between treatment group comparisons at week 12. As part of the main study, smoking is assessed by self-report and is biochemically confirmed (CO < 5) weekly after quitting, at EOT (week 8), and at the 12- week follow-up. Food intake is measured by three consecutive days of 24-hour food recalls at baseline and 4- 8- 12- week post-TQD.

3.4 Study Duration – Parent (Main) Study

Recruitment/enrollment began in earnest in September 2016 and will continue for ~44 months. We anticipate that ~340 participants will have completed the study by December 2020. We estimate that it will take ~30 weeks/8 months for a participant to complete the entire study.

3.5 Study Duration- Neuroimaging Sub-Study

Recruitment/enrollment for the neuroimaging sub-study is anticipated to begin in late July or August 2017 and will continue for ~18 months. We estimate that up to 50 participants will have completed the neuroimaging sub-study by ~April 2019. We estimate that it will take ~10 weeks/2.5 months for a participant to complete the neuroimaging sub-study (i.e. both fMRI scans).

3.6 Study Duration- Microbiome Sub-Study

Recruitment/enrollment for the microbiome sub-study is anticipated to begin in late September or October 2019 and will continue for ~8-10 months. We estimate that up to 60 participants will have completed the microbiome sub-study by ~July 2020.

4 CHARACTERISTICS OF THE STUDY POPULATION

4.1 Target Population

Participants will be treatment-seeking males and females between the ages of 18-65 who report smoking at least 5 cigarettes/day for at least the past 6 months.

4.2 Accrual - Parent (Main) Study

We will enroll ~625 participants (i.e., provide consent) to achieve a sample of ~340 who enter treatment (i.e., randomized) and remain enrolled through the 26-week assessment. To achieve this goal over a ~44-month enrollment period, we will complete 55 telephone screens monthly to obtain 25 smokers who meet criteria. Of these smokers, we expect ~15 to attend the Intake Visit, and ~10 per month to be confirmed eligible to be randomized after completing the Intake Visit.

In order to increase retention throughout the study we will: (1) educate subjects about the benefits of participation and the knowledge gained from the study; (2) schedule sessions at times convenient for participants; (3) provide reminder calls; and (4) provide payment for completion of all study visits. As is the convention in smoking cessation trials, smokers who are lost to follow-up will be included in the analysis and counted as smokers.

4.3 Accrual - Neuroimaging Sub-Study

Eligible participants enrolled in the parent study who meet the fMRI eligibility criteria will have the opportunity to participate in the neuroimaging sub-study. We estimate that in order to achieve a sample of ~50 participants who complete the neuroimaging sub-study with usable data, we will need to enroll ~75 participants into the sub-study. Accrual estimates are based on our extensive experience conducting neuroimaging studies and specifically recruitment of fMRI subsamples from larger clinical trials. To ensure retention, we: (1) educate subjects about the benefits of participation and knowledge gained from the study; (2) schedule sessions at convenient times; (3) provide reminder calls; and (4) provide incentives for completion of sessions and assessments. This accrual rate is highly feasible based on our prior work.

4.4 Accrual – Microbiome Sub-Study

Eligible participants enrolled in the parent study who meet the microbiome eligibility criteria will have the opportunity to participate in the microbiome sub-study. We estimate that in order to achieve a sample of up to 60 participants who complete the microbiome sub-study with usable data, we will need to enroll ~75 participants into the sub-study as our retention rate through 12 weeks is ~80%. To ensure retention in the sub-study, we will offer monetary incentives for providing stool samples per the study instructions.

4.5 Inclusion Criteria

1. Male and female treatment-seeking smokers who are between 18 and 65 years of age and self-report smoking at least 5 cigarettes (menthol and/or non-menthol) per day for at least the last 6 months.
2. Plan to live in the area for the duration of the study (i.e. ~30 weeks/8 months).
3. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the combined consent and HIPAA form.
4. Smokers who wish to make a permanent quit attempt in the next 1-2 months (treatment-seeking), because our prior work suggests that motivated subjects are more sensitive to medication effects on smoking behavior. Using a scale from 0 to 100 (100, being extremely interested), subjects must rate their interest in quitting smoking within the next 1-2 months greater than 50.
5. Able to communicate fluently in English (i.e. speaking, writing, and reading).
6. Participants will provide a Urine Cotinine sample that is present at a sensitivity of 100 ng/mL at the Intake Visit. If deemed safe by the Principle Investigator under federal and University guidelines, participants will provide a Carbon Monoxide (CO) breath test reading greater than or equal to 5 parts per million (ppm) at the Intake Visit

4.6 Exclusion Criteria

Subjects who present and/or self-report with the following criteria will not be eligible to participate in the study:

<u>Smoking Behavior</u>

1. Regular use of nicotine containing products other than cigarettes (e.g. chewing tobacco, snuff, snus, cigars, e-cigs, etc.). Participants agreeing to abstain from using nicotine containing products other than cigarettes and the study-provided TN for the duration of trial will be considered eligible.
2. Current enrollment or plans to enroll in another research and/or smoking cessation program over the duration of the study (i.e. ~30 weeks/8 months).
3. Anticipated use (within the next ~30 weeks/8 months) of any nicotine substitutes and/or smoking cessation treatments/medications unless provided through the study.
4. Participant who provide a Urine Sample that is absent of Cotinine at a sensitivity of 100 ng/mL at the Intake Visit. If deemed sage by the Principle Investigator under federal and University guidelines, if participants provide a CO breath test reading less than 5 ppm at Intake will not be eligible to participate in the study.

<u>Alcohol and Drug</u>

1. History of substance abuse (other than nicotine) in the past 12 months and/or currently receiving medical treatment for substance abuse. Counseling and support groups (e.g. Alcoholics Anonymous and Narcotics Anonymous) will not be considered medical treatment for the purposes of this protocol.
2. Current alcohol consumption that exceeds 25 standard drinks/week.
3. Breath alcohol reading (BrAC) greater than .000 at the Intake Visit for participants who complete an in-person consent. Participants who completed a virtual REDCap consent

and telephone Consent Discussion will not be required to complete a BrAc reading for the safety of the participant and the staff members due to the risk of exposure to COVID-19. Further, a BrAc reading is unnecessary in this case because the participant would have consented at a different time prior to coming into the center for their Intake Visit.

4. A positive urine drug screen (UDS) for cocaine, opiates, amphetamines, methamphetamines, phencyclidine (PCP), ecstasy (MDMA), barbiturates, benzodiazepines, methadone, and/or oxycodone at the Intake Visit.

Medical

1. Women who are pregnant, breast feeding, or planning a pregnancy over the duration of the study period. Women must agree to use an adequate form of contraception or abstain from sexual intercourse for the duration and for at least one month after the end of the study.
2. Current treatment of cancer or diagnosed with cancer (except basal or squamous-cell carcinoma not treated with chemotherapy and/or radiation) in the past 6 months.
3. Poorly controlled, brittle, or pump-dependent Type I diabetes.
4. Current peptic ulcer bleeding.
5. Allergy to adhesive tape.
6. Skin problems or sensitivities. Eligibility will be evaluated on a case-by-case basis by the Study Physician.
7. Active hepatitis or poorly controlled kidney and/or liver disease.
8. Uncontrolled hypertension (systolic blood pressure [SBP] greater than 159 and/or diastolic blood pressure [DBP] greater than 99; see Blood Pressure Procedures under Screening/Covariates).
9. History of abnormal heart rhythms, tachycardia, and/or cardiovascular disease (e.g. stroke, angina, heart attack) may result in ineligibility. These conditions will be evaluated on a case-by-case basis by the Study Physician.
10. History of epilepsy or seizures. Eligibility will be evaluated on a case-by-case basis by the Study Physician.
11. Serious or unstable disease within the past 6 months. Notable diseases will be evaluated on a case-by-case basis by the Principal Investigator and/or the Study Physician.
12. Any impairment including, but not limited to, visual, physical, and/or neurological impairments preventing proper completion of the study procedures. Notable impairments will be evaluated on a case-by-case basis by the Principal Investigator and/or the Study Physician.
13. Low or borderline intellectual functioning – determined by receiving a score of less than 75 on the Shipley Institute of Living Scale (SILS), which correlates with the Wechsler Adult Intelligence Scale-Revised (WAIS-R) Estimated IQ Test.
14. Applicable food allergies or disorders:
    - Galactosemia [a]
    - Notable milk allergy (lactose intolerant participants may proceed unless they experience severe symptoms) [b]
    - Notable soy allergy [c]
    - Peanut allergy [d]

[a,b,c] Boost® Original Very Vanilla Nutritional Shake: Contains milk and soy ingredients. Suitable for Lactose Intolerance.
[a,b,c,d] M&M's® (Milk Chocolate): Contains milk and soy ingredients (MAY CONTAIN PEANUTS)
Lay's® Classic Potato Chips (Gluten Free): Potatoes, vegetable oil, and salt

Psychiatric

1. Current diagnosis of major depression. Subjects with a history of major depression, in remission for 6 months or longer (may be stable on antidepressant medications), are eligible.
2. Lifetime history of a suicide attempt.
3. Lifetime history of schizophrenia, psychosis, and/or bipolar disorder.

Medication

Current use or recent discontinuation (within the last 14 days) of:

1. Smoking cessation medication (e.g., Zyban, Wellbutrin, Wellbutrin SR, Chantix).
2. Anti-psychotic medications.
3. Prescription stimulants (e.g., Provigil, Ritalin, Adderall).
4. Systemic steroids.

Current use of:

5. Nicotine replacement therapy (NRT).
6. Heart medications such as digoxin, quinidine, and nitroglycerin.

Daily use of:

7. Benzodiazepines and/or Barbiturates.
8. Opiate-containing medications for chronic pain.
9. Inhaled corticosteroids.

Subjects will be instructed to refrain from using any study prohibited drugs/medications (both recreational and prescription) throughout their participation in the study. After final eligibility is confirmed, subjects who report taking contraindicated medication(s) over the course of the study period may only remain eligible if the Study Physician and Principal Investigator determine that the contraindicated medication(s) do/did not significantly impact the study design, data quality, and/or subject safety and welfare. Subjects are permitted to take necessary prescription medications not included within the exclusion list during the study.

General Exclusion

1. Past, current, anticipated, or pending enrollment in another research program over the study period that could potentially impact subject safety, study data, and/or the study design as determined by the Principal Investigator and/or Study Physician.
2. Any medical condition, illness, disorder, adverse event (AE), or concomitant medication that could compromise participant safety or significantly impact study performance as determined by the Principal Investigator and/or Study Physician. Subjects may be deemed ineligible for any of the aforementioned reasons at any point throughout the study, as well as during the initial telephone screen.
3. Significant non-compliance with protocol and/or study design as determined by the Principal Investigator and/or Study Physician. Subjects may be deemed ineligible at any point throughout the study.
4. Subjects failing to complete an in-person Baseline Visit will be excluded (no Principal Investigator determination required).

fMRI Exclusion Criteria

The following fMRI exclusion criteria are only pertinent to the neuroimaging sub-study sample – subjects who are not eligible to complete the neuroimaging sub-study may still complete the parent study if appropriate per protocol:

1. History of claustrophobia.
2. Being left-handed.
3. Lifetime history of stroke.
4. Having a cochlear implant or wearing bilateral hearing aids.

5. History of notable head trauma. Although notable head trauma is typically defined as being knocked unconscious for a period of three minutes or longer, notable head trauma will be evaluated on a case-by-case basis by the appropriate personnel prior to an eligibility decision considering exceptions can be made based on the nature and severity of the trauma.
6. History of brain or spinal tumor.
7. Pacemakers, certain metallic implants or objects, or presence of metal in the eye as contraindicated for MRI.
8. Any circumstances (e.g., exclusionary metal implants, certain dental work, and/or physical impairments) and/or conditions that may interfere with MRI and MRI-related study visit procedures. All potential exclusionary circumstances and/or conditions will be evaluated on a case-by-case basis by the appropriate personnel prior to an eligibility decision.
9. History of gunshot wounds. Injuries from BB guns will be evaluated on a case-by-case basis by the appropriate personnel prior to an eligibility decision.
10. History of epilepsy and/or recurrent or uncontrolled seizures.
11. Weight greater than 250 lbs at Intake Visit or self-reported at phone screen. If a participant weighs less than or equal to 250 lbs at Intake, but presents with a weight greater than 250 lbs at either Scan 1 and/or Scan 2, the participant may be permitted to proceed with the scan as long as the participant's weight does not exceed 300 lbs.
12. A positive urine drug screen (UDS) for cocaine, opiates, amphetamines, methamphetamines, phencyclidine (PCP), ecstasy (MDMA), barbiturates, benzodiazepines, methadone, and/or oxycodone at either Scan Visit. Depending on the substance and circumstances, a subject may be deemed ineligible for the main study at the discretion of the Principal Investigator as well.
13. A BrAC greater than 0.010 at either Scan Visit. Depending on the circumstances, the Principal Investigator may permit the participant to remain in the sub-study and reschedule a neuroimaging scan to another day.

Microbiome Sub-Study Exclusion Criteria

The following exclusion criteria only pertain to inclusion within the microbiome sub-study. Subjects who are not eligible to participate the microbiome sub-study may still complete the parent study if appropriate per protocol.

Current use or recent discontinuation (within the last 21 days) of the following medications:

1. Antibiotics (topical antibiotics are permissible)
2. Antifungals (topical antifungals are permissible)
3. Antivirals (topical antivirals are permissible)
4. Psychotropics

Once a participant is confirmed as eligible for the microbiome sub-study at the Intake Visit, they may remain enrolled in the sub-study even if they report the use of sub-study exclusionary medications listed above. This caveat is in recognition that the impact of certain medications may be unknown until the completion of analyses.

4.7 Vulnerable Populations

Children, pregnant women, fetuses, neonates, or prisoners are not included in this research study. Educationally or economically disadvantaged persons are included but not solely targeted for recruitment. Because of our recruitment efforts for this study, it is possible that University of Pennsylvania employees and students may be invited to participate. Status of participation in the current study will be independent of the participant's work or school activities.

4.8 Subject Recruitment

Participants may be recruited from television, radio, internet advertisements, social media, newspaper, flyers, transit posters, referrals, and/or from our database of previous participants who have agreed to be re-contacted for future studies. All advertising materials will be submitted to the UPENN IRB for approval prior to distribution/posting. Interested participants will first complete a telephone screen to assess their initial eligibility. Participants who are initially eligible will be screened against our registration database to confirm that they are not currently participating in another research study at our Center and have not previously reported a condition or circumstance that would make them ineligible for the current study. . **If the PI deems it is safe for the participant and research staff, based upon federal and University COVID-19 guidance,** then eligible research participants will be invited to the center to complete an Intake Visit during which they will be presented with the IRB approved Informed Consent Form in-person and have their final eligibility confirmed.

Prior to the Principle Investigator's approval, those participants who remain initially eligible at phone screen will then be invited to review the entire IRB approved Informed Consent Form virtually via REDCap to minimize the participants time spend in the research center during the COVID-19 pandemic. After reviewing the IRB approved Informed Consent Form, the participant will complete a telephone Consent Discussion with a member of the research staff. After the participant has had the opportunity to have their questions answered, signed the consent, and agreed to participate, the participant will complete a Shipley Institute of Living Scale (SILS) virtually via REDCap. Participants whose estimated IQ is 75 according to the WAIS-R conversion based upon the exam will be deemed Ineligible per the Exclusion Criteria above. Participants whose WAIS-R estimated IQ is at least a 75 based upon the Shipley will then be invited to the center to have their final eligibility confirmed at an Intake Visit. Early Withdrawal of Subjects

4.8.1 When and How to Withdraw Subjects

Subjects are free to withdraw from the study at any time. Subjects may be deemed "ineligible" at any time per the exclusion criteria listed in section 4.5. No follow-up data collection is required for participants who withdraw or are deemed ineligible throughout the study.

5 Study Drug (Transdermal Nicotine [TN])

5.1 Description

The medication utilized in this trial, open-label TN (NicoDerm® CQ® - Clear), will be used in accordance with FDA-approved labeling except participants smoking at least 10 cigarettes per day will only utilize TN for 8 weeks instead of 10 weeks. This shortened dosing regimen is neither a significant change nor does it pose an increased risk to subjects. The results of this trial will not be reported to the FDA to support a new drug indication. This study is not intended to support a significant change in the advertising for TN. This study will not involve a route of administration or dosage level, use in a subject population, or other factor that would significantly increase the risks (or decrease the acceptability of the risks) associated with TN.

5.2 Treatment Regimen

All eligible subjects receiving Study Physician approval will receive 8 weeks of open-label TN patches (NicoDerm® CQ® - Clear). TN treatment will commence week 0 (TQD) and conclude week 8 (EOT). All participants who report smoking 10 or more cigarettes a day at the Intake Visit will receive the 21mg dose for the first 4 weeks, 14mg for the subsequent 2 weeks, and

7mg for the final 2 weeks. Participants who report smoking 5-9 cigarettes a day at the Intake Visits will receive the 14mg dose for 6 weeks and the 7mg dose for the final 2 weeks.

Participants included within the neuroimaging sub-study will be instructed to remove their 7mg TN patch prior to entering the MRI environment during their Scan 2 Visit only (no patch worn during the pre-treatment scan). Participants will be provided with a loose, sealed 7mg (NicoDerm® CQ® - Clear) patch to reapply after exiting the MRI environment.

5.3 Preparation and Packaging of Study Drug

A trained member of the research team will distribute TN patches (NicoDerm® CQ® - Clear) per a standard distribution schedule. For every two weeks of treatment, participants will receive an unopened box of TN patches (n=14) of the appropriate dose, as well as two extra "loose" patches of the appropriate dose in an opaque storage bag. A label containing the participant's study I.D. number, dosage (mg), counselor name and phone number, Study Physician name and phone number, as well as additional safety and storage instructions will be secured to all unopened boxes and loose storage bags of TN prior to distribution.

5.4 Subject Compliance Monitoring

TN patch adherence data will be collected and recorded at every applicable time point. The research staff will collect all unused patches and attempt to ensure the amount of unused patches matches the patch adherence data. All discrepancies will be explored and recorded as appropriate.

5.5 Receiving, Storage, Dispensing and Return

5.5.1 Receipt of Drug Supplies

TN will be shipped directly to the CIRNA. Upon receipt of TN to the CIRNA, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. Study staff will verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable TN in a given shipment will be documented in the study files.

5.5.2 Storage

TN patches will be stored in a double locked location (i.e., in a locked cabinet in a locked room) at room temperature (68-77°F). Temperature will be verified and recorded on a temperature log at regular intervals. Any readings outside the desired range will be documented and the temperature adjusted accordingly.

5.5.3 Dispensing and Reconciliation of Study Drug

The appropriate amount of TN patches will be distributed to eligible participants at week -1 (PQ), week 1 (Mid-Tx.1), and week 4 (Mid-Tx. 3) according to a standardized patch distribution schedule. TN dosage (see section 5.2: Treatment Regimen) will be dependent on the participant's daily smoking rate reported at the Intake Visit.

Regular study drug reconciliation will be performed to document drug dispensed, drug used, and drug returned. This reconciliation will be logged on both a subject-specific drug reconciliation form maintained in the subject's study chart, as well as an overall study drug accountability form. Returned or expired TN will be secured and logged into onsite quarantine. UPENN Environmental Health & Radiation Safety (EHRS) will make periodic pick-ups of the quarantined TN for incineration. Records of EHRS pick-ups will be maintained in the study files.

5.5.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug received, drug consumed, and drug remaining. This reconciliation will be logged on a study completion drug reconciliation form, signed and dated. Any notable discrepancies will be investigated, resolved, and documented prior to destruction of unused study drug. Unused or expired TN will be secured and logged into onsite quarantine. UPENN EHRS will make a final pick-up of the quarantined TN for incineration. A record of EHRS final pick-up will be maintained in the study files.

6 Study Procedures

6.1 Telephone Eligibility Screen

Individuals interested in study participation will be screened by a qualified member of the research team to determine initial study eligibility. If the participant meets preliminary eligibility, they will be invited to schedule an Intake Visit at which their final eligibility will be confirmed.

6.2 In-Center Visits

6.2.1 Visit Reminders

Participants will typically receive study visit reminders 24 – 48 hours prior to their scheduled study visits via phone call, email, and text message (if applicable). Prior to the Follow-Up visits, participants may receive reminder letters, emails, and/or phone calls up to two weeks in advance.

6.2.2 Intake Visit (~Week -3)

During the Intake Visit (Duration: ~3 hours) participants will:

1. In order to limit the number of people in the research center and their study visit duration during the COVID-19 pandemic, the PPT will complete a virtual IRB approved informed consent and HIPAA presentation via REDCap where all the study procedures and institutional policies will be reviewed prior to coming in to complete the remainder of their Intake Visit until the Principle Investigator **deems it is safe for the participant and research staff to conduct in-person consenting, based upon federal and University COVID-19 guidelines.**
    - The participant will be required to view the entire combined informed consent and HIPAA form prior to signing the document. Upon completing their reading of the consent, the PPT will be offered the opportunity to indicate if they have any questions and a staff member will call the participant to complete a consent discussion via the telephone.All participant questions will be recorded and answered as appropriate after which the combined informed consent and HIPAA form will be completed (virtually signed and dated) by both the participant and a qualified member of the research team.
    - The staff will print a signed copy of this completed record and provide a copy to the participant and save a copy for our records.
    - If the Principle Investigator grants approval, the participant may complete the informed consent and HIPAA presentation in-Person to review the study procedures and institutional policies. All of their questions will be recorded and answered as appropriate after which the combined informed consent and HIPAA form will be completed (signed and dated) by both the participant and a qualified

member of the research team. The participant and the research study will each maintain a completed copy for their records.

2. Confirm the accuracy of information (i.e. name, address, phone number, email [if applicable], date of birth, age, gender, ethnicity, and race) provided during the initial Telephone Eligibility Screen.
3. Complete a UDS (at least 30ml [two tablespoons] of urine). The UDS will assess the use of any study-prohibited medications/recreational drugs (See Key Exclusionary Criteria; Alcohol and Drug).
    - Participants who test positive for any exclusionary medications or recreational drugs per this protocol will be deemed ineligible..
4. Female participants only: Self-administer a CLIA-waived urine pregnancy test.
    - Female participants are informed that the participation of pregnant women in this study is prohibited and that if they believe they are pregnant they should withdraw from the study immediately.
5. The Participant's urine sample will be used to administer a Urine Cotinine screen with a sensitivity of 100 ng/mL to confirm their smoking status in the absence of a CO reading in order to maintain the safety of the research staff and research participant during the ongoing COVID-19 pandemic.
    - Participants who fail to indicate the presence of cotinine of at least 100 ng/mL in their urine will be deemed ineligible per this protocol.
6. May perform a BrAC assessment to control for alcohol consumption if participants completed an in-person consent and the Principle Investigator deems it is safe for researchers and research participants according to federal and University guidelines.
    - Participants with a BrAC greater than 0.000 at Intake Visit will be ineligible.
7. May perform a CO breath assessment and self-report smoking behavior over the past 24 hours to control for prior tobacco exposure the Principle Investigator deems it is safe for researchers and research participants according to federal and University guidelines
    - Participants with a CO reading less than 5 ppm will be deemed ineligible.
8. Complete a blood pressure measurement (See Blood Pressure Procedures under Screening/Covariates section)
9. Complete height and weight measurements.
10. Complete a Medical History Form with a member of the research team to review for applicable contraindications previously listed under the Inclusion and Exclusion Criteria sections.
11. **Participants who meet the microbiome sub-study eligibility criteria at telephone screening and elect to participate in the microbiome sub-study only**: Complete a Microbiome Medication Review form
    - Participants who do not meet the microbiome eligibility criteria will be deemed ineligible for the microbiome sub-study. Participants may still participate in the parent study per protocol.
12. Complete a baseline concomitant medication review (if applicable).
13. **Participants who meet neuroimaging eligibility criteria at telephone screening and elect to participate in the neuroimaging sub-study only**: Complete an fMRI Medical History Form, Magnet Safety Form, and Emergency Contact Form.

- Participants who do not meet the fMRI eligibility criteria will be deemed ineligible for the neuroimaging sub-study. Participants may still participate in the parent study per protocol.

14. Complete the Shipley Institute of Living Scale IQ test in-person if the Principle Investigator deems it is safe for researchers and research participants according to federal and University guidelines, or virtually via REDCap
    - Participants earning less than an estimated WAIS-R IQ score of 75 will be deemed ineligible and will not be invited to complete the remainder of their Intake Visit in-person if they completed their exam on REDCap Participants who complete their SILS via REDCap will not receive travel reimbursement, because they did not travel to the center.
15. Complete paper and pencil questionnaires:
    - Demographics
    - Smoking History/Nicotine Dependence (FTND)
    - ETOH History
    - Cigarette Brand Form (staff will record cigarette brand information)
    - Program Referral Form
16. Complete the Individualized Food (Pre-FCQ-S) task. Foods rated least appetizing (n=20) and foods rated most appetizing (n=20) will be selected to create personalized food cue stimuli for the Food Cue-Induced Craving (FCQ-S) task administered over the course of the study.
17. Select either the salty (Lay's® Classic Potato Chips) or sweet (M&M's® [Milk Chocolate]) snack food as the snack to "work for" in the Relative Reinforcing Value of Food (RRVF) task over the course of the study.
18. Schedule the Baseline Visit with a member of the research team. Participants who are eligible for the neuroimaging sub-study and elect to participate may schedule their Scan 1 Visit as well. All participants will schedule the remainder of their visits and dietary recalls at the conclusion of the Baseline Visit.

6.2.3 Baseline Visit (~Week -2)

Participants are asked to not consume food or caffeine for 4 hours prior to the beginning of the visit. During the Baseline Visit (Duration: ~3 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Depression Symptoms (CES-D)
    - Hedonic Capacity (SHAPS)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
    - Habitual Food Craving (FCQ-T)
    - Eating Behavior (DEBQ)
    - Eating Inventory-Disinhibition (EI-D)
    - Weight Concerns

5. Immediately prior to the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
6. Participants will be provided with a Boost® Nutritional Drink to consume prior to completing the laboratory tasks.
7. Complete computerized laboratory tasks:
    - Food Cue-Induced Craving (FCQ-S)
    - Food Reward
    - Food Reinforcement (RRVF)
8. Immediately after the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
9. Participants will complete a standardized 30-minute wait period. During the wait period, participants will complete the paper and pencil questionnaire:
    - Alternative Reinforcers (freq, enjoy) (PES)
10. Complete Session 1 of assigned counseling condition (BAS+ or SC) and schedule the remainder of their study visits and 24-hour dietary recalls. Participants will receive a copy of their study calendar at this time.

6.2.4 Pre-Quit Visit (~Week -1)

During the Pre-Quit Visit (Duration: ~2 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Participants who required multiple blood pressure readings during the Intake Visit will complete an additional blood pressure reading to reconfirm eligibility to receive TN as scheduled (See Blood Pressure Procedures under Screening/Covariates).
5. Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
6. Complete Session 2 of assigned counseling protocol (BAS+ or SC) and receive TN per a standardized distribution schedule (see section 6.5.1: Treatment; Transdermal Nicotine).

6.2.5 Target Quit Day Visit (Week 0)

During the Target Quit Day Visit (Duration: ~1.5 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:

- Side Effect Checklist (SEC)
- Adverse Events Form (Open-Ended AEs)
- Withdrawal Symptoms (MNWS)
- Smoking Urges/Craving (QSU-B)
- Positive & Negative Affect (PANAS)
- Physical Activity (PAR)

5. Complete Session 3 of assigned counseling protocol (BAS+ or SC), as well as provide TN progress and adherence information.

6.2.6 Mid-Treatment 1 Visit (Week 1)

Participants are asked to not consume food or caffeine for 4 hours prior to the beginning of the visit. During the Mid-Treatment 1 Visit (Duration: ~3 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
   - Side Effect Checklist (SEC)
   - Adverse Events Form (Open-Ended AEs)
   - Withdrawal Symptoms (MNWS)
   - Smoking Urges/Craving (QSU-B)
   - Positive & Negative Affect (PANAS)
   - Physical Activity (PAR)
5. Immediately prior to the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
6. Participants will be provided with a Boost® Nutritional Drink to consume prior to completing the laboratory tasks.
7. Complete computerized laboratory tasks:
   - Food Cue-Induced Craving (FCQ-S)
   - Food Reward
   - Food Reinforcement (RRVF)
8. Immediately after the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
9. Participants will complete a standardized 30-minute wait period. During the wait period, participants will complete the paper and pencil questionnaire:
   - Alternative Reinforcers (freq, enjoy) (PES)
10. Complete Session 4 of assigned counseling condition (BAS+ or SC), provide TN progress and adherence information, and receive TN per a standardized distribution schedule (see section 6.5.1: Treatment; Transdermal Nicotine).

6.2.7 Mid-Treatment 2 Visit (Week 2)

During the Mid-Treatment 2 Visit (Duration: ~1.5 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).

3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
    - Alternative Reinforcers (freq, enjoy) (PES)
5. Complete Session 5 of assigned counseling condition (BAS+ or SC), as well as provide TN progress and adherence information.

6.2.8 Mid-Treatment 3 Visit (Week 4)

Participants are asked to not consume food or caffeine for 4 hours prior to the beginning of the visit. During the Mid-Treatment 3 Visit (Duration: ~3 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
    - Habitual Food Craving (FCQ-T)
    - Eating Behavior (DEBQ)
    - Eating Inventory-Disinhibition (EI-D)
5. Immediately prior to the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
6. Participants will be provided with a Boost® Nutritional Drink to consume prior to completing the laboratory tasks.
7. Complete computerized laboratory tasks:
    - Food Cue-Induced Craving (FCQ-S)
    - Food Reward
    - Food Reinforcement (RRVF)
8. Immediately after the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
9. Participants will complete a standardized 30-minute wait period. During the wait period, participants will complete the paper and pencil questionnaire:
    - Alternative Reinforcers (freq, enjoy) (PES)
10. Complete Session 6 of assigned counseling condition (BAS+ or SC), provide TN progress and adherence information, receive TN per a standardized distribution schedule (see section 6.5.1: Treatment; Transdermal Nicotine), and confirm the 24-hour dietary recall (#2) schedule.

6.2.9 Mid-Treatment 4 Visit (Week 6)

During the Mid-Treatment 4 Visit (Duration: ~1.5 hours) participants will:

1. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
5. Complete Session 7 of assigned counseling condition (BAS+ or SC) and provide TN progress and adherence information.

6.2.10 End of Treatment Visit (Week 8)

The End of Treatment Visit will be scheduled after patch treatment has concluded. Participants are asked to not consume food or caffeine for 4 hours prior to the beginning of the visit. During the End of Treatment Visit (Duration: ~3 hours) participants will:

1. Perform a CO breath assessment or provide a urine sample for a cotinine assessment answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
    - Withdrawal Symptoms (MNWS)
    - Smoking Urges/Craving (QSU-B)
    - Positive & Negative Affect (PANAS)
    - Physical Activity (PAR)
    - Habitual Food Craving (FCQ-T)
    - Eating Behavior (DEBQ)
    - Eating Inventory-Disinhibition (EI-D)
5. Immediately prior to the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”
6. Participants will be provided with a Boost® Nutritional Drink to consume prior to completing the laboratory tasks.
7. Complete computerized laboratory tasks:
    - Food Cue-Induced Craving (FCQ-S)
    - Food Reward
    - Food Reinforcement (RRVF)
8. Immediately after the laboratory tasks, participants will be asked to rate, “How hungry are you right now?” using a scale from 0 “not at all” to 10 “extremely.”

9. Participants will complete a standardized 30-minute wait period. During the wait period, participants will complete the paper and pencil questionnaire:
    - Alternative Reinforcers (freq, enjoy) (PES)
10. Complete Session 8 of assigned counseling condition (BAS+ or SC), provide TN progress and adherence information, and confirm the 24-hour dietary recall (#3) schedule.

6.2.11 COVID-19 Quitting Experiences Survey

Active enrolled participants who concluded their patch treatment and completed their EOT Visit on or after 3/30/2020 may be contacted to complete a survey intended to capture their experiences quitting smoking during the COVID-19 pandemic. Participants will be asked to provide verbal consent for this data collection when contacted, as well as, for any future contact needed to clarify any information collected during the survey. Participants will also be asked to provide verbal consent for the recording of the survey. If a participant does not consent to completing the survey or being recorded, the survey will not be administered and the call will be ended. The call will be recorded using a recording service, e.g. TapeACall. All recordings will be stored on our secure server, and only staff will have access to those files. Recording will be used to ensure participant responses were captured exactly when the data is reviewed. Staff will complete the survey via REDCap (i.e. verbally administering questions and recording responses), and no other personal information will be collected. Participants will complete the survey during the End of Treatment or Follow-Up 1 Visit when possible.

The survey will include 24 questions recommended by the NIH for research pertaining to COVID-19: 11 questions related to smoking behavior and the pandemic formulated by the staff, and the PHQ-2 and GAD-2 measures for depression and anxiety symptoms (4 questions total). If a participant scores a 3 or higher on either the PHQ-2 or GAD-2 measure, they will be offered resources for coping strategies.

6.2.12 Follow-Up Visits 1 and 2 (Week 12 and Week 26)

During Follow-Up Visits 1 and 2 (Duration: ~30 minutes) participants will:

1. Perform a CO breath assessment or provide a urine sample for a cotinine assessment, answer combustible marijuana items, and complete a concomitant medication review.
2. Provide daily smoking rate (timeline follow back [TLFB]).
3. Have weight measured.
4. Complete paper and pencil questionnaires:
    - Adverse Events Form (Open-Ended AEs)
    - Physical Activity (PAR)
5. If a participant reports not smoking (not even a puff of a cigarette) for at least the 7 days prior to the Follow-Up Visit without the aid of NRT, the participant will be asked to provide a 5ml saliva sample used to assess cotinine levels and verify abstinence from smoking.
6. Confirm the 24-hour dietary recall (Week 12 [#4] and Week 26 [#5]) schedule.

6.2.13 fMRI Scan Visits 1 & 2 (~Week -2 and ~Week 7-8)

Participants who are both eligible and elect to participate in the neuroimaging sub-study will complete two neuroimaging sessions: Scan 1 will occur prior to the Baseline Visit (~week -2) and Scan 2 will occur during the final week of nicotine patch therapy (~week 7-8). Prior to each scan, participants will be instructed to fast overnight (beginning at ~10pm) until after the

completion of their fMRI scanning visit the following day. The fast period does not include a Boost® Nutritional Drink (Vanilla) that will be provided during the scanning visit to standardize prior food intake and remove deprivation to increase sensitivity to changes in food reward. Since participants are asked to fast overnight, all scanning sessions will be scheduled to occur in the morning. However, a scan may be completed in the afternoon at the discretion of the Principal Investigator due to extenuating circumstances. Additionally, participants will be instructed not to consume caffeine for 4 hours prior to the beginning of the visit in order standardize satiety. Including the 1-hour fMRI scan, scanning visits are expected to last ~3 hours in duration. If a scanner hardware/software malfunction occurs and data is unable to be collected, an fMRI scan(s) may be rescheduled at the discretion of the Principal Investigator.

At both scanning visits participants will:

1. Complete a urine drug screen (at least 30ml [two tablespoons] of urine). The urine drug screen will assess the use of any study-prohibited medications/recreational drugs (See Key Exclusionary Criteria; Alcohol/Drugs). Participants who test positive for any study-prohibited medications/recreational drugs will not be permitted to complete the neuroimaging sub-study and may be deemed ineligible for the main study at the discretion of the Principal Investigator.
2. Self-administer a CLIA-waived urine pregnancy test (female participants only). Participants who believe that they may be pregnant are instructed to discontinue participation in both the neuroimaging sub-study and parent study at this time.
3. Perform a BrAC assessment to control for alcohol consumption.
    - Participants with a BrAC greater than 0.010 will be ineligible to complete the neuroimaging sub-study (may remain in the parent study) unless the Principal Investigator permits the participant to reschedule the neuroimaging scan to another day.
4. Perform a CO breath assessment, answer combustible marijuana items, and complete a concomitant medication review.
5. Self-report smoking behavior over the past 24 hours to control for prior tobacco exposure and provide their daily smoking rate (timeline follow back [TLFB]).
6. Have weight measured.
    - Participants weighing over 300 lbs will be ineligible to complete the neuroimaging sub-study. However, participants may remain enrolled in the main study.
7. **SCAN 2 ONLY:** Provide TN progress and adherence information.
8. **SCAN 2 ONLY**: Complete paper and pencil questionnaires:
    - Side Effect Checklist (SEC)
    - Adverse Events Form (Open-Ended AEs)
9. Complete a magnet safety form.
    - Participants who do not meet the fMRI eligibility criteria will be deemed ineligible for the neuroimaging sub-study. Participants may still participate in the parent study per protocol.
10. Complete a set of abbreviated practice tasks similar to those that will be administered during the fMRI scan, as well as an individualized calibration task to determine the amount of food (i.e. “Food Value Number”) that is equivalent to $0.25 in order to calculate the standard portion size of a participant’s preferred snack food (i.e. chips or M&Ms®) that may be earned on a given trial within the in-scanner Food

Reinforcement/Choice task. Participants will be expected to demonstrate an understanding of the tasks and the response device prior to the fMRI scan.

11. Be provided with a Boost® Nutritional Drink and water.
12. Be escorted to the imaging facility at either the Hospital of the University of Pennsylvania or the Stellar Chance building for the brain-scanning portion of the session.
13. **SCAN 1 ONLY**: Participants will smoke one of their own cigarettes (~30 min. before initiating fMRI) in a designated smoking area outside of the building where the fMRI scanner is located in order to standardize the timing of cigarette exposure for all participants.
14. **SCAN 2 ONLY:** Participants will be instructed to remove their TN patch prior to entering the MRI environment. Participants will be provided with a loose, sealed 7mg (NicoDerm® CQ® - Clear) patch to reapply after exiting the MRI environment.
15. Undergo a 1-hour fMRI scan adhering to the procedures and Table 1 below:

Scanning Protocol/Data Acquisition: MRI is performed in a 1-hour session, which, in our experience, participants tolerate easily without discomfort or excessive motion. Prior to entering the scanning area, an approved MRI Technician or MRI User will review the participant's magnet safety form and will confirm that the participant has removed all metal and/or objects containing magnetic strips from their persons. Once the participant is approved to enter the scanning area, the participant will be placed supine in the scanner, wearing earplugs to muffle noise, and fitted with adjustable foam cushions to limit head motion. If a participant requires corrective lenses for vision, but does not have contact lenses, a set of plastic glasses that are approved for use in an MRI scanner will be provided to him/her. Participants will be provided with an emergency squeeze ball, so that they may interrupt the scan and/or be removed from the scanner if necessary. Head fixation will be assured through a foam-rubber device mounted on the headcoil.

Image acquisition is performed on a 3T Siemens Tim-Trio scanner with a FDA approved head coil. A T1-weighted multi-echo magnetization-prepared, rapid acquisition gradient echo (MPRAGE) structural image is acquired using standard parameters at 1mm resolution. This MPRAGE is used for functional image co-registration and transformation into standard template space. Resting BOLD fMRI will be obtained followed by the three task paradigms.

TABLE 1: fMRI Session

~Duration (mins.)	Activity
5	Structural Scans (localizer, MPRAGE)
10	Resting State BOLD fMRI
15	Food Reinforcement/Choice [a]
12	Working Memory [a,b]
10	Food Cue Reactivity [a]
2	B0 Map **if time permits**

Total Time in Scanner: ~54 minutes
[a] fMRI tasks will be presented in a fixed order
[b] Stimuli (Form A/B) will be counterbalanced within subject

Task stimuli will be rear projected to the center of the visual field using a PowerLite® 7300 Video projector system (Epson American, Inc., Long Beach, CA) and viewed through a mirror mounted on the head coil. Participants will use a fiber optic response pad (FORP™ Current Design, Inc., Philadelphia, PA) made of non-ferromagnetic components. This MR-compatible button-box is used to record task responses and reaction times. Pulse and respiration are recorded as an index of physiological arousal during the task and to statistically reduce the effects of physiological noise in the fMRI data.

Resting State fMRI: Whole-brain functional MR images will be acquired while subjects rest with eyes closed over 39 axial, interleaving, 4-mm sections by means of a gradient-echo echo planar imaging sequence (150 volumes; echo time/repetition time, 27/2000 milliseconds; flip angle, 80°; field of view, 220 x 220 mm; image matrix, 64x64).

Task fMRI: BOLD images will be acquired using a whole-brain, single-shot gradient-echo (GE) echo-planar imaging (EPI) sequence with the following parameters: TR/TE=2000/30 ms, flip angle 90°, field of view (FOV)=192 mm, matrix = 96X96, isotropic voxel resolution 2.5 mm, slice

thickness/gap=2.5/0mm, 38 slices axial-oblique parallel to AC-PC. In ongoing studies we find these parameters produce robust signal in ventral brain regions including ventral striatum and orbital/ventral prefrontal cortex with little distortion or signal loss.

Food Cue Reactivity: The task will present (5 sec) 20 pictures of food rated (at Intake) as most appetizing and 20 pictures of food rated (at Intake) least appetizing, and 20 pictures of a water glass. Participants will be instructed to imagine tasting and eating the pictured food for as long as the food is presented. Order of presentation is randomized and the task consists of 60 events separated by an inter-stimuli interval (fixation point) ranging from 2-11 sec (mean 5.5 sec). As a secondary measure, food craving will be assessed prior to and after completion of the scan. The food craving assessment is the 3-item "desire" subscale of the reliable and valid state version of the Food Craving Questionnaire (FCQ-S). Total task time: ~10 minutes.

Food Reinforcement/Choice: Participants choose between a standard low effort monetary reward and either (on separate trials) a food or monetary reward of higher value and effort. Each trial presents a choice between a standard reward (27 button clicks for $0.25) or exerting greater effort to earn a larger reward. On half of the trials, larger rewards will be $0.50 (2 x standard value). In the remaining trials the larger reward will be double the calibrated snack portion (e.g., 20 M&Ms). Effort for more valuable rewards will vary from 27 to 775 responses in 25 log10 steps. Pairs of visual stimuli depicting the two options appear for 4.5 sec followed by a 3-14 sec inter-trial interval. Each pairing is presented twice making 50 trials for each class. Left/right position of stimuli is randomized, as is trial type (monetary vs. food; effort levels). To increase motivation to choose food and monetary reward, participants are told they will complete the work and receive the outcome of 4 randomly selected food and monetary (maximum $2.00) trials. Total task time: ~15 minutes.

Working Memory: We will use a 12-minute visual N-back task validated for fMRI studies of smokers. A total of 20 fractals (target-foil ratio 1:3) are presented for each condition (i.e., 0-back, 2-back, 3-back), and each condition (60 sec block) is repeated 3 times in pseudo-random block design experiment. Each fractal is displayed for 500ms followed by 2500ms inter stimuli interval. Total task time: ~12 minutes.

16. When all scans are completed, participants will be removed from the MRI scanner and be asked to complete a brief ~5-minute "work" task (i.e. clicking on a target a certain amount of times) based on their selections during the in-scanner Food Reinforcement/Choice task on a laptop computer.

17. Participants will receive the appropriate reward(s) (food and/or monetary) per the post-scan work task, as well as receive compensation for time and travel per the study compensation schedule. At the end of the session, participants will be escorted to the exit of the imaging facility where s/he will be free to leave.

6.3 Microbiome Sub-Study

Participants who are both eligible and elect to participate in the microbiome sub-study will be instructed to collect and return stool samples to the Center at **Week -2 (Baseline Visit), Week 4 (Mid-Tx 3 Visit)**, and **Week 8 (End of Treatment Visit after completing TN treatment)**. Participants will be asked about their consumption of yogurt, probiotics, and prebiotics at **the Baseline Visit, Mid-Tx 3 Visit**, and **End of Treatment Visit.** Participants will be asked to answer a few questions regarding their antibiotic use over the past 6 months at the **Baseline Visit** as well.

Participants will receive a stool sample collection kit and detailed instructions (written and verbal) about how to complete the collection of their stool sample at the in-Center visit prior to the visit when which they are scheduled to return their sample. Ideally, stool samples will be

collected and returned to the Center in person within 24 hours (up to ~36 hours is permissible) of the designated visit. If a participant is unable to attend a visit at which a stool sample collection kit is distributed, the stool sample collection kit may be mailed to the participant if deemed appropriate by the Principal Investigator. If a participant is unable to attend a visit at which a stool sample is scheduled to be returned or they are physically unable to provide a stool sample within 24-36 hours of their designated sample-return visit, the participant may return their stool sample to the Center on a date after the designated sample-return visit. The Principal Investigator will determine the window of time that a participant will be permitted to return their stool sample after the designated sample-return visit on a case-by-case basis.

In addition to take-home collection instructions and Stool Sample Collection Form, participants will receive the following supplies in a stool sample collection kit: Stool collection bucket with a lid, a frame into which the bucket fits for collection, a large, sealable gallon bag, cold packs and a bag for ice, small styrofoam cooler, nitrile gloves, absorbent, and a shipping box. Participants will complete each stool sample collection at their residence. Participants will be instructed to place the stool collection bucket in the commode, which allows for separation of urine and feces, and keeps the feces separated from the toilet water. Participants will place a lid on the bucket (labeled with participant ID number and designated time point) and record the date and time of sample collection on the Stool Sample Collection Form**.** The sealed bucket will then be secured in the large bag with absorbent material. The bagged sample should then be placed in the cooler with several cold packs and/or bags of ice.

If the participant is unable to return their stool sample to the Center in person, a cardboard shipping box may be included in the stool sample collection kit for shipping directly to the CHOP Microbiome Center or CIRNA. To prepare for shipping, the Styrofoam cooler is placed in the cardboard shipping box with a biohazard label for shipping by courier. The participant will then call the courier service for pick-up of the sample for overnight delivery to the CHOP Microbiome Center or CIRNA per a standardized procedure.

Stool samples returned to the CIRNA will be immediately refrigerated and hand delivered within a cooler to the CHOP Microbiome Center by the research staff as soon as possible. Once the stool sample is aliquot for metagenomics analyses, the appropriate amount of sample will be delivered to the Penn Metabolomics Core for metabolomics analyses per a standardized procedure.

6.3.1.1 Metagenomics

Metagenomics will be used to examine changes in the composition of the gut microbiome. Shotgun libraries will be generated from 1 ng of DNA using the NexteraXT kit (Illumina, San Diego, CA). Libraries will be sequenced on the Illumina HiSeq using 2x125 bp chemistry in High Output mode. Extraction blanks and DNA free water will be included to empirically assess environmental and reagent contamination. Laboratory-generated mock communities consisting of DNA from *Vibrio campbellii*, *Cryptococcus diffluens*, and Lambda phage will be included as positive sequencing controls. Bioinformatics analysis will be carried out with the Sunbeam metagenomics analysis pipeline, which Dr. Bittinger and staff helped write (https://doi.org/10.1101/326363). Adaptor sequences will be removed from reads using Trimmomatic. Host DNA will be identified by alignment to the human genome (version hg38), and low-complexity sequences will be removed using the Komplexity tool, which is distributed with Sunbeam pipeline. Taxanomic classification will be performed using Kraken and a reference database of bacterial genomes available from NCBI. Sequence reads will be assembled into contigs using the ensemble method in MEGAHIT. The bioinformatics analysis will include taxonomic and gene function abundances for bacteria, fungi, and double stranded

DNA viruses. For classification of bacterial genes, we will utilize the KEGG reference database, for which the Center maintains an institutional license.

6.3.1.2 Metabolomics

Metabolomics will assess the downstream changes in neuroactive products of gut bacteria. Metabolites will be extracted from stool samples according to well-established, validated methods for tissue-based metabolomics and readied for untargeted LC/MS metabolomics. Reversed-phase C18 chromatography to retain and separate medium polarity to nonpolar metabolites will be performed on a Thermo Scientific Ultimate 3000 UHPLC using a Waters Acquity BEH C18 column (2.1 mm x 150 mm, 1.7 µm). HILIC chromatography to retain highly polar metabolites, not retained by reversed-phase C18 chromatography, will be performed on the Ultimate 3000 using a Waters Acquity BEH-NH2 column (2.1 mm x 150 mm, 1.7 µm). The Ultimate 3000 UHPLC will be coupled to a novel Thermo Fisher Scientific Orbitrap ID-X mass spectrometer scanned from 75-1000 Da at a resolution of 70,000. Compound Discoverer (Thermo Fisher Scientific, San Jose, CA) will be used to process the LC/MS metabolomics data to identify metabolites and determine statistical differences between study groups.

6.4 Table 2. Study Measures and Time Points

Time Point *(Scan 1 and 2 are completed as part of the fMRI/neuroimaging sub-study only)*	**Intake**	**Scan 1**	**BSL**	**PQ**	**TQD**	**Mid-Tx 1**	**Mid-Tx 2**	**Mid-Tx 3**	**Mid-Tx 4**	**Scan 2**	**EOT**	**FU**	
												1	**2**
Counseling Session (BAS+ or SC)			**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**		**S8**		
Week	**-3**	**-2**	**-2**	**-1**	**0**	**1**	**2**	**4**	**6**	**~7-8**	**8**	**12**	**26**
TREATMENT													
BAS+ or SC			X	X	X	X	X	X	X		X		
Transdermal Nicotine (TN)					X	X	X	X	X	X	X		
SCREENING/COVARIATES													
Urine Drug Screen	X	X								X			
Urine Pregnancy Screen (if applicable)	X	X								X			
Urine Cotinine	X										X	X	X
Breath Alcohol (BrAC)	X	X								X			
Height	X												
Blood Pressure *(*only if multiple BP assessed at Intake)*	X			X*									
Medical History Form	X												
Microbiome Medication Review *(Microbiome Sub-Study Only)*	X												
fMRI Medical Hx. Form *(fMRI Sub-Study Only)*	X												
Magnet Safety Form *(fMRI Sub-Study Only)*	X	X								X			
Emergency Contact Form *(fMRI Sub-Study Only)*	X												
Demographics	X												
ETOH History	X												
Smoking History/Nicotine Dependence (FTND)	X												
Cigarette Brand Form	X												
Program Referral Form	X												
Shipley Institute of Living Scale (SILS)	X												
Hunger Scale *(**administered before and after lab tasks)*			X**			X**		X**			X**		
Physical Activity (PAR)			X	X	X	X	X	X	X		X	X	X
Habitual Food Craving (FCQ-T)			X					X			X		
Eating Behavior (DEBQ)			X					X			X		
Eating Inventory-Disinhibition (EI-D)			X					X			X		
Positive & Negative Affect (PANAS)			X	X	X	X	X	X	X		X		
Withdrawal Symptoms (MNWS)			X	X	X	X	X	X	X		X		
Smoking Urges/Craving (QSU-B)			X	X	X	X	X	X	X		X		
Hedonic Capacity (SHAPS)			X										
Treatment Adherence (Counseling/TN)			X	X	X	X	X	X	X	X	X		
Side Effects Checklist (SEC)				X	X	X	X	X	X	X	X		
Adverse Events Form (Open-Ended AEs)				X	X	X	X	X	X	X	X	X	X
Carbon Monoxide (CO)	X	X	X	X	X	X	X	X	X	X	X	X	X
Combustible Marijuana Items		X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Antibiotics Form *(Microbiome Sub-Study Only)*			X										
Yogurt and Pro/Prebiotics Form *(Microbiome Sub-Study Only)*			X					X			X		
MODERATORS													
Weight Concerns			X										
Depression Symptoms (CES-D)			X										
Gender	X												
MECHANISMS													
Individualized Food (Pre-FCQ-S)	X												
Preferred Snack Choice (Pre-RRVF)	X												
Food Cue-Induced Craving (FCQ-S)			X			X		X			X		
Food Reward			X			X		X			X		
Food Reinforcement (RRVF)			X			X		X			X		
Alternative Reinforcers (freq, enjoy) (PES)			X			X	X	X			X		
MICROBIOME VARIABLES													
Stool Sample *(Microbiome Sub-Study Only)*			X					X			X		
OUTCOMES													
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Food Intake (24-Hour Dietary Recalls)			X					X			X	X	X
Smoking Rate Timeline Follow Back (TLFB)		X	X	X	X	X	X	X	X	X	X	X	X
Smoking Cessation (CO)					X	X	X	X	X	X	X	X	X
Smoking Cessation (Saliva Cotinine)												X	X

fMRI Tasks *(fMRI Sub-Study Only)*		X								X			

6.5 Description of Study Measures

6.5.1 Treatment

Standard Smoking Cessation Counseling (SC): The initial session (**week -2, Baseline [pre-quit]**) begins with a review of smoking and quitting history, reasons for smoking and quitting, triggers for smoking, obtaining social support for quitting, and self-monitoring of smoking. The 2nd session (**week -1, Pre-Quit**) will focus on the management of smoking triggers, slip recovery and relapse prevention. In preparation for the TQD (following week) the counselor will discuss the role of TN in withdrawal symptom management, directions on how to use TN, and the schedule (e.g., 4 weeks 21mg, 2 weeks 14mg, 2 weeks 7mg; (NicoDerm® CQ® - Clear). The 3rd session (**week 0, TQD**) will focus on the quit day experiences given that the participant will have been instructed to quit the morning of this session. The benefits of quitting will be emphasized along with evaluating and reinforcing progress with cessation, managing triggers, and relapse prevention. Adherence to nicotine patch use recommendations will be emphasized. Sessions 4-8 (**weeks 1, 2, 4, 6 and 8 post-TQD**) will include evaluating and reinforcing progress with cessation, reviewing the management of triggers to smoke, relapse prevention, and problem solving difficulties and challenges such as slips. Overeating and weight gain are common concerns reported during smoking cessation treatment. Per convention, SC will address these concerns through standard recommendations to consume low-calorie snack foods, drink water, eat nutritious meals, and exercise (e.g., NCI's Clearing the Air). SC will not include skills to shape the use of these suggestions.

Behavioral Activation Intervention (BAS+): SC is incorporated into BAS+ in an additive design to cover best practice guidelines for smoking cessation. The goal of the BAS+ is to maintain a level of overall reward after cessation by structuring and enhancing opportunities for reinforcement to: (1) ensure that not smoking is as reinforcing as smoking; and (2) prevent an over-reliance on food as a substitute reinforcer for smoking so that PCWG does not precipitate smoking relapse. The unique treatment components of BAS+ include facilitating the identification and engagement in a variety of rewarding activities (other than eating and not associated with smoking) and maximizing the enjoyment derived from these rewarding activities. As such, this intervention should benefit all smokers trying to quit, not just those participants who feel that preventing PCWG is meaningful.

In the initial session **(week -2, Baseline**), the counselor will discuss why smokers easily substitute snacking for cigarettes after smoking cessation, and the consequences for weight gain and continued abstinence. The treatment rationale for promoting smoking cessation and preventing PCWG will focus on structuring a variety of reinforcing activities to promote a more rewarding nonsmoking lifestyle; one that has many rewarding options besides food. By replacing the pleasure previously derived from smoking, the participant will miss cigarettes less, rely less on calorie-dense snacks, gain less weight, and be less likely to return to smoking. The counselor will introduce daily activity monitoring and the identification of values and goals in several life domains (e.g., family and intimate relationships, employment/career, hobbies, recreation, spirituality). The daily activity monitor promotes awareness of time spent in important and or enjoyable activities and their association with smoking. The identification of values and life goals will be used to identify rewarding activities consistent with helping one live according to his or her values. Participants will be assisted in identifying rewarding activities (i.e., alternative reinforcers, not linked to smoking) in important life domains through a life activities checklist.

In the second session **(week -1, Pre-Quit)**, the goals and experiences with daily activity monitoring will be reviewed, noting the time spent in important and/or enjoyable activities. Using the life values and goals self-identified in the 1st session, participants will set daily goals for selecting and engaging in these activities (e.g., reinforcer sampling) to begin to increase alternative reinforcers prior to quitting smoking. Through an activity hierarchy (easiest to most

difficult to achieve), the counselor will ensure that the planned activities are realistic, available and accessible. Participants will self-select activities to ensure that they engage in activities that they feel are rewarding. Participants will keep track of their daily progress with the daily activity monitoring form. Participants will rate the enjoyment/importance of the self-selected activities and note if any of the activities increased craving or were not enjoyable. Smoking is a daily reinforcer and this task will facilitate thinking about alternative reinforcers to smoking and snacking that are linked to their goals and values. Participants will be counseled to use caution (or forego) and employ smoking cessation strategies when engaging in activities (i.e., friends who smoke, alcohol use), that increase the risk of smoking.

Given that nicotine may increase the pleasure derived from available reinforcers, BAS+ will include a component to build skills to enhance the pleasure derived from reinforcers. These types of "savoring" skills are effective at promoting long-term increases in positive emotions and life enjoyment. At the end of each day, while rating the enjoyment level of planned activities or experiences, participants will mentally re-visit three things that made these activities enjoyable. These behavioral skills also serve to enhance the relative value of smoke-free and snack-free activities.

In addition to quit day events, the third session **(week 0, TQD)** will focus on daily activity monitoring, engagement and planning. Based on successful engagement in selected activities, new activities will be added for monitoring in with the goal of progressively adding more challenging activities. Reward enhancement goals and experiences also will be reviewed. The rationale and importance of alternative reinforcers for quitting smoking and minimizing weight gain will be emphasized. Based on experience, the participant will tailor their planned daily activities to link remaining abstinent and gaining less weight to the broader context of their values and goals.

Sessions 4-8 **(weeks 1, 2, 4, 6 and 8 post-TQD).** Key components will be discussed including identifying and engaging in rewarding activities, and enhancing the reward derived from the activities. The sessions will focus on reviewing successes and problem-solving difficulties, such as whether slips/relapse to smoking or over-snacking coincided with absent alternative reinforcers or reinforcers not consistent with remaining abstinent. Relapse will be considered within the context of available alternative reinforcers and the participant's specific values and life goals. Individuals who relapse will be directed to set another quit date and to re-evaluate their selection of alternative reinforcers. Some activities may not be as rewarding as originally thought or they may be associated with smoking or snacking. Additionally, participants will be encouraged to revisit overall values and goals if difficulties arise. The rationale for these core behavior activation practices will be reiterated from the standpoint of remaining abstinent, lessening PCWG and achieving value-driven life goals.

Transdermal Nicotine (TN): All participants who report smoking 10 or more cigarettes a day will receive the 21mg dose for the first 4 weeks, 14mg for the subsequent 2 weeks, and 7mg for the final 2 weeks. Participants who report smoking 5-9 cigarettes a day will receive the 14mg dose for 6 weeks and the 7mg dose for the final 2 weeks. TN treatment will commence **week 0 (TQD)** and conclude **week 8 (EOT)**. Any severe adverse reactions or significant side effects of TN will be medically evaluated by the Study Physician as per this protocol. TN use by individuals experiencing notable side effects will be monitored, adjusted, or discontinued as needed. If the 21mg patch proves too strong for some participants, and they begin to experience signs of nicotine overdose, the 14mg and/or the 7mg patch will be provided instead (all other study procedures will remain standard). The appropriate amount of nicotine patches (NicoDerm® CQ® - Clear) will be distributed to participants at week -1 (PQ), week 1 (Mid-Tx.1), and week 4 (Mid-Tx. 3) according to a standardized patch distribution schedule. Regardless of whether a participant lapses or relapses, patch treatment will only be distributed for a total of 8 weeks. If a participant is unable to attend an in-person visit or does not have enough patches to continue with their treatment schedule, additional supplies of nicotine patches may be mailed (authorized parcel service) or picked up by the participant if approved by the Principal Investigator. If TN is mailed, the participant must be available to sign for the delivery and receive the TN patches

personally. Participants who discontinue TN due to a medical event may continue with the study sans TN.

6.5.2 Screening/Covariates

Urine Drug Screen: A urine sample (~20-30ml) will be collected at the Intake Visit and both neuroimaging scans (neuroimaging sub-study only) to conduct a urine drug screen. The urine drug screen indicates whether the subject has recently taken any of the following recreational drugs or medications: THC, cocaine, opiates, amphetamines, methamphetamines, phencyclidine (PCP), ecstasy (MDMA), barbiturates, benzodiazepines, methadone, tricyclic antidepressants, and/or oxycodone. Participants with a positive urine drug screen for any substance listed above other than THC or tricyclic antidepressants at the Intake visit will be deemed ineligible. A urine sample that doesn't register a temperature reading of at least 90 degrees Fahrenheit will not be considered a valid sample. In an effort to remain CLIA-compliant, results from urine drug screen will not be shared with participants. Participants will be informed that the testing is for research purposes only and that they will be informed of their eligibility status, but not of the specific testing results. In order to document inclusion/exclusion criteria for regulatory purposes, results of the urine drug screens (test cup) will be retained in research charts and in an electronic research record within our local data management system (Access). These results are not and will not be entered into a participant's electronic medical record.

Participants who test positive for any study-prohibited medications/recreational drugs at either neuroimaging scan (neuroimaging sub-study only) will not be permitted to complete the neuroimaging sub-study and may be deemed ineligible for the main study at the discretion of the Principal Investigator.

Urine Cotinine Test: While the University of Pennsylvania continues to limit the number of personnel allowed in research spaces and requires all researchers and research participants to wear a mask, at the Intake Visit, the End of Treatment (EOT), and Follow-Up 1 and 2 Visits, a urine sample of (~20-30 mL) will be collected to conduct a urine cotinine screen. The urine cotinine screen indicates the presence of cotinine in the urine, which is a product of nicotine metabolism with a sensitivity of 100 ng/mL. Participants will be informed of their eligibility status at Intake, but not the specific testing results. The Urine Cotinine Test will be used to confirm their spoking behavior at the subsequent EOT and Follow-Up 1 and 2 visits. This measure will be used to confirm a participant's smoking status until the Principle Investigator believes it is safe per federal and University guidelines for the participants and staff to resume CO readings to confirm smoking status. In order to document inclusion/exclusion criteria and smoking behavior data for analytic and regulatory purposes, results from the urine cotinine screen will be retained in research charts and in an electronic research record within our local data management system (Access). These results are not and will not be entered into a participant's electronic medical record.

Urine Pregnancy Test: At the Intake Visit and both neuroimaging scans (neuroimaging sub-study only), female participants will be supplied with a simple, CLIA-waived hCG pregnancy test strip and a urine sample cup (participants may use the urine drug screen cup if desired). Participants will then be instructed to self-administer the pregnancy test and be told that if they are (or believe they may be) pregnant they should not participate in the research study. The participants will inform the study staff if they are able to continue participation after they have reviewed the results of the pregnancy test. Participants will be informed that there is no penalty for discontinuing participation at this point in the visit and that they will still receive travel reimbursement for the visit.

Breath Alcohol Concentration (BrAC):): **If the PI deems it is safe for the participant and research staff, based upon federal and University COVID-19 guidance,** participants who complete an in-person IRB Approved Consent and HIPAA form will complete a BrAC assessment at the Intake Visit and both neuroimaging scans (neuroimaging sub-study only). Participants will be made aware of the BrAC assessment prior to all applicable visits and asked to avoid alcohol and alcohol-based products (e.g. mouthwash, breath spray, etc.) the evening and morning before the Intake Visit and both neuroimaging scans (neuroimaging sub-study only). The BrAC monitor is a handheld device that uses a disposable mouthpiece and reports the concentration of alcohol in exhaled breath. Any reading greater than 0.000 indicates alcohol consumption within the last 14 hours. Participants with a BrAC greater than 0.000 at the Intake Visit will be deemed ineligible. Participants with a BrAC greater than 0.010 at either neuroimaging scan will be ineligible to complete the neuroimaging sub-study (may remain in the main study) unless the Principal Investigator permits the participant to reschedule the neuroimaging scan to another day. Participants testing positive for breath alcohol with a reading equal to or greater than .08 (the legal driving limit in Pennsylvania) or who are visibly impaired will be instructed not to drive themselves home after the visit. If a participant needs to use a phone to call for a safe ride home, an office telephone will be made available to the participant.

Height: Research staff will collect and document participant height utilizing a mounted stadiometer at the Intake Visit.

Blood Pressure Procedures:

Intake Visit**.** Participants presenting with elevated blood pressure (SBP greater than 159 mmHg and/or DBP greater than 99 mmHg) at the Intake Visit will have a second blood pressure reading taken after a 10-minute rest period in which the participants will be instructed to sit comfortably. If, after the second reading:

- A participant presents with a SBP reading less than 160 mmHg and a DBP less than 100 mmHg, s/he will be ELIGIBLE for the study.
- A participant presents with a SBP reading greater than 159 mmHg and/or DBP greater than 99 mmHg, s/he will be INELIGIBLE for the study.
- A participant presents with a SBP reading greater than 190 mmHg and/or DBP greater than 110 mmHg, s/he will be deemed INELIGIBLE for the study and instructed to seek immediate medical attention (e.g. Emergency Room or Walk-in Clinic).

After the first or second blood pressure reading, if a participant presents with a SBP between 151-159 mmHg and/or DBP between 91-99 mmHg, the research staff will inform the participant that they may have mild, Stage I, hypertension and advise they consult with a physician. The participant will remain ELIGIBLE for the study.

Pre-Quit Visit. Participants who required a second blood pressure at the Intake Visit (i.e. the first measurement was a SBP greater than 159 mmHg and/or a DBP greater than 99 mmHg) will have their blood pressure reassessed at Pre-Quit. If a participant presents with elevated blood pressure (SBP greater than 159 mmHg and/or DBP greater than 99 mmHg), staff will take an additional blood pressure reading after a 10-minute rest period in which the participant will be instructed to sit comfortably. If, after the second reading:

- A participant presents with a SBP reading less than 160 mmHg and a DBP less than 100 mmHg, s/he will be ELIGIBLE to receive TN patches as scheduled.
- A participant presents with a SBP greater than 159 mmHg and/or DBP greater than 99 mmHg, the participant will be required to obtain written permission (a formal letter will be provided by the study team) from their physician in order to receive TN patches. If TN patches are not distributed at the Pre-Quit Visit for this reason, the participant may still participate in the study and receive counseling only. The participant may receive TN patches after the receipt of an approved letter signed by the participant's physician.
- A participant presents with a SBP greater than 190 mmHg and/or DBP greater than 110 mmHg, s/he will be instructed to seek immediate medical attention (e.g. Emergency Room or Walk-in Clinic). The participant may still participate in the study and continue and receive counseling, but they will not have the option to receive TN patches.

After the first or second blood pressure reading, if a participant presents with a SBP between 151-159 mmHg and/or DBP between 91-99 mmHg, the research staff will inform the participant that they may have mild, Stage I, hypertension and advise they consult with a physician. The participant will remain eligible for the study and receive TN patches as scheduled.

Missed Pre-Quit Visit. For participants requiring a second blood pressure at the Intake Visit (i.e. first measurement was SBP greater than 159 mmHg and/or DBP greater than 99 mmHg) who miss their scheduled Pre-Quit Visit, a blood pressure measurement will occur at the next in-person visit the participant attends. Blood pressure will be collected in accordance with the procedures (Pre-Quit Visit) listed above. TN patches will not be distributed until the participant presents a blood pressure reading within the established eligibility range (post Intake).

Hypotension. If a participant presents with a SBP less than 90 mmHg at either the Intake Visit or Pre-Quit Visit, blood pressure should be retaken after a 10-minute rest period. If after the second reading the participant still presents with a SBP less than 90 mmHg, the Study Physician will be consulted to determine study eligibility and/or if TN patches should be distributed.

Medical History Form: A medical history form (led by the research staff) will be completed to review for applicable contraindications previously listed under Key Inclusion/Exclusion Criteria at the Intake Visit.

Microbiome Medication Review: Participants who elect to participate in the microbiome sub-study and met the initial microbiome sub-study eligibility criteria at phone screen will be asked about their use of medications that would be considered contraindicated for participation. Those reporting the use of an exclusionary medication will be deemed ineligible to participate in the microbiome sub-study.

fMRI Medical History Form: Participants who are eligible to potentially complete the neuroimaging sub-study and met the initial fMRI eligibility criteria at phone screen will be asked to complete a separate fMRI medical history form to review for applicable contraindications previously listed under Key Inclusion/Exclusion Criteria.

Magnet Safety Form: Participants participating in the neuroimaging sub-study will complete a standard magnet safety form provided by the Department of Radiology at the Hospital of the University of Pennsylvania at the Intake visit and before entering the scanner at each fMRI scan. The magnet safety form assesses a history of specific prosthesis, surgical implants, and a variety of other MRI contraindications.

Emergency Contact Form: Participants participating in the neuroimaging sub-study form will provide emergency contact information in the event of an emergency at the fMRI scanner.

Demographics, Smoking History, Cigarette Brand, and Nicotine Dependence (FTND): Standard questionnaires will be administered at the Intake Visit to collect the following data: demographics, age at smoking initiation, current smoking rate, previous quit attempts, and own cigarette brand information (Cigarette Brand Form). The Fagerstrom Test for Nicotine Dependence (FTND) will also be administered. The FTND is a 6-item, self-report measure of nicotine dependence derived from the Fagerstrom Tolerance Questionnaire. The FTND scale has satisfactory internal consistency (Cronbach's alpha = .64) and high test-retest reliability (r =.88).

ETOH History: An ETOH history questionnaire will be administered at the Intake Visit and will assess alcohol consumption over the past 7 days.

Program Referral Form: Participants who are in contact with current or past study participants may share information exclusive to their assigned counseling condition (BAS+/SC). To avoid this situation and protect blind integrity, participants will be asked to complete a Program Referral Form at the Intake Visit in which they will identify if they know someone currently enrolled or someone who has previously completed the trial. Those acquainted with current or previous participants may be randomized to the same counseling condition (BAS+/SC) at the Principal Investigator’s discretion.

Shipley Institute of Living Scale (SILS): All participants will complete the SILS via REDCap prior to coming into the center to complete the remainder of their Intake Visit unless it is deemed safe

for the participants and the staff to spend larger durations of time in the center under federal and University COVID-19 safety guideliens.The SILS is a self-administered test designed to assess general intellectual functioning in adults and adolescents and to aid in identifying cognitive impairments. The scale consists of two subtests, a 40-item vocabulary test and a 20-item test of abstract thinking. The total administration time is 20 minutes (10 minutes per subtest). The REDCap Exam will score the test according to the SILS instructions and WAIS-R conversion. The SILS correlates with the Wechsler Adult Intelligence Scale-Revised (WAIS-R) Estimated IQ Test; those participants earning less than an estimated WAIS-R IQ of 75 will be ineligible. The SILS is considered a highly reliable assessment tool with good total score internal consistency (Cronbach's alpha= .92).

Hunger Scale: Immediately prior to and after completion of computerized laboratory tasks, participants will be asked to rate, "How hungry are you right now?" using a scale from 0 "not at all" to 10 "extremely."

Physical Activity (PAR): Physical activity will be assessed with the 7-day Physical Activity Recall (PAR). The PAR has excellent test-retest reliability (r=0.81) and validity (r = 0.72 heart rate monitor). It is a widely used measure of habitual activity. The PAR provides data on frequency, intensity, duration, and kilocalories expended.

Habitual Food Cravings (FCQ-T): Habitual food cravings will be assessed with the trait version of the Food Craving Questionnaire. This valid and reliable 39-item measure asks participants to indicate on 6-point scale the frequency of food craving (never to always).

Eating Behavior (DEBQ): The Dutch Eating Behavior Questionnaire will assess ***restrained eating*** (10 items, tendency to restrict food intake), ***external eating*** (10 items, tendency to eat in response to food related cues) and ***emotional eating*** (13 items, tendency to eat in response to emotions). These scales are associated with food craving and response to food cues. Participants indicate on a Likert-style scale how often each item is applicable to them (0= not relevant; 1 = never to 5=very often).

Eating Inventory-Disinhibition (EI-D): Disinhibition will be measured with a subscale (16 items) of the Eating Inventory that is thought to reflect responsivity to environmental food cues, and internal cues, and linked to food reinforcement and weight change.

Positive and Negative Affect (PANAS): The Positive and Negative Affect Schedule (1-week frame of reference), a 20-item Likert-format self-report questionnaire, will be used to measure positive mood and negative mood. The two subscales have 10 items each, are internally consistent in both nonpsychiatric and psychiatric samples (α = .79 to 91), and exhibit good convergent and discriminant validity.

Withdrawal Symptoms (MNWS): The Minnesota Nicotine Withdrawal Scale - Revised version (MNWS-R) will measure ***withdrawal symptoms*** (n=15) associated with quitting smoking. Participants will rate the intensity of their symptoms on the following scale: 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe and a summary score will be calculated. The MNWS with a 1-week frame of reference will be utilized throughout the trial.

Smoking Urges/Craving (QSU-B): The well-validated and reliable 10-item brief Questionnaire of Smoking Urges will assess ***craving*** for cigarettes. The QSU-B utilizes a "right now" frame of reference.

Hedonic Capacity (SHAPS): Hedonic capacity will be measured with the 14-item Snaith-Hamilton Pleasure Scale (SHAPS). Participants will rate the degree of enjoyment they would get from hypothetical activities typically experienced as enjoyable. The SHAPS loads strongly on

measures of anhedonia (r = .92), is internally consistent (α = .87), stable over time, and has adequate convergent and discriminant validity in clinical and nonclinical populations.

Treatment Adherence: Treatment adherence is defined as attending 6 of 8 counseling sessions and using 6 or more patches on average per week. TN adherence will be assessed and recorded at every in-person visit over the treatment period.

Side Effects Checklist (SEC): Participants will complete a SEC at all in-person visits after the Baseline Visit. The SEC will assess the severity of side effects that may be TN related and experienced by participants in the study (See section 7.1: Potential Study Risks; Transdermal Nicotine). These reports will be formally documented as **anticipated (expected)** AEs whether or not the event(s) is ultimately deemed related or not related to the use of TN in this study. The reporting period for each assessment will inquire about any side effects experienced since the last in-person visit. Items will be rated by participants utilizing the following severity scale: 0 (None=No Concerns), 1 (Mild=Side effect does not interfere with usual daily activities), 2 (Moderate= Side effect does interfere with some activities), and 3 (Severe=No normal activities are possible). See section 8.2.1: AE Collection Methods for further details.

Adverse Events Form (Open-Ended AEs): Participants will be asked an open-ended question about any symptom or medical event that may be related to their study participation not included on the SEC form at all in-person visits after the Baseline Visit. These events will documented as **unanticipated (unexpected)** AEs unless they are otherwise outlined in the protocol and/or consent form (i.e. related to withdrawal, assessments, etc.). The reporting period for each assessment will inquire about any event(s) or symptom(s) experienced since the last in-person visit. If a participant reports a symptom or medical event, they will be asked to rate the severity of the event utilizing the following severity scale: 0 (None=No Concerns), 1 (Mild=Issue does not interfere with usual daily activities), 2 (Moderate=Issue does interfere with some activities), and 3 (Severe=No normal activities are possible). See section 8.2.1: AE Collection Methods for further details.

Carbon Monoxide (CO): **If the PI deems it is safe for the participant and research staff, based upon federal and University COVID-19 guidance,** CO will be measured at the Intake Visit and all subsequent in-person visits to confirm smoking status. The CO monitor is a handheld device that uses a disposable mouthpiece, reports CO in parts per million (ppm), and takes about 3-5 minutes to administer. In order to remain eligible and confirm smoking status at the Intake Visit, a participant must present with a CO greater than or equal to 5 ppm**.**

Combustible Marijuana Items: Participants will be asked if they smoked combustible marijuana in the past 24 hours. If yes, the participant will be asked how many hours ago they last used. Information collected on combustible marijuana may be considered a confounding variable during data analysis in relation to CO level.

Concomitant Medications: At the Intake Visit, participants will be asked to list all medications (prescription or non-prescription) and NRTs currently taken and/or recently discontinued (within the past 14 days) as a baseline collection. All information will be collected on a Concomitant Medication Log that will be maintained within the participant's study chart. At every subsequent in-person visit, participants will be asked if they have taken any additional medications (prescription or non-prescription), NRTs, and/or changed the dosage or stopped taking any previously reported medications since their last in-person visit. Participants who report taking contraindicated medication(s) over the course of the study period may only remain eligible if the Study Physician and/or Principal Investigator determine that the contraindicated medication(s) did not impact the study design, data quality, and/or participant safety and welfare.

Antibiotics Form: Participants who are participating in the microbiome sub-study will be asked about their antibiotic use over the past six months at the Baseline Visit.

Yogurt and Pro/Prebiotic Form: Participants who are participating in the microbiome sub-study will be asked about their consumption of yogurt and pro/prebiotics use over the past four weeks at the Baseline Visit (wk-2), Mid-Tx. 3 Visit (wk4), and End of Treatment Visit (wk8).

6.5.3 Moderators

Weight Concerns: Weight concerns associated with quitting smoking will be measured with a reliable (α=.87) and valid 6–item scale. Responses to the 6 items range from 1 (not at all) to 10 (very much). Scores are the average of responses to all items.

Depression Symptoms (CES-D): The Center for Epidemiologic Studies Depression Scale is a 20-item Likert-style scale used to assess depressive symptomatology. This scale has high internal consistency (r=.85-.90) and has been shown to correlate with clinical ratings of the severity of depression. In our previous research, CES-D scores have correlated significantly with self-medication smoking and nicotine dependence.

Gender: Gender (self-identification) will be assessed via self-report during the initial telephone screen and reconfirmed in-person at the Intake Visit.

6.5.4 Mechanisms

Individualized Food (Pre-FCQ-S): In preparation for the food cue-induced craving task, participants will rate how appetizing they find pictures of food (e.g., cupcakes, fruits, vegetables) at the Intake Visit. Foods rated least appetizing (n=20) and foods rated most appetizing (n=20) will be selected to create personalized food cue stimuli.

Preferred Snack Choice (Pre-RRVF): Participants will select either the salty (Lay's® Classic potato chips) or sweet (M&M's®) snack food as the snack to "work for" when completing the RRVF task over the remainder of the study.

Food Cue-Induced Craving (FCQ-S): Food cue-induced craving will be assessed using a personalized food cue exposure paradigm, whereby pictures of foods rated least appetizing (L, n=20), most appetizing (M, n=20), and a picture of a glass of water (W, n=20) will be presented to participants seated at a computer screen. Participants will be instructed to imagine tasting and eating the pictured food for as long as the image is presented. The images will be presented in six blocks. Each block consists of image presentation (10 images, 8 sec duration) and craving assessment. The M, L, and W food cues will be presented in one of two sequences (i.e., MWLMWL or LWMLWM) counterbalanced across participants. Before the task and after each block, participants will complete the state version of the Food Craving Questionnaire, Desire Subscale (FCQ-S). The 3 items (desire to eat, craving, urge to eat) will be tailored to ask about the M, L, and W images on a 5-point scale (strongly disagree to strongly agree). Personalized cues can maximize craving induction and produce greater increases in subjective craving and objective indices of cue reactivity than standard cues. Reactivity to palatable food images is associated with body mass and weight gain. The Food Cue-Induced Craving task will be administered at the Baseline (Week -2), Mid.-Tx 1 (Week 1), Mid.-Tx 3 (Week 4), and End of Treatment (Week 8) Visits.

Food Reward: Food reward will be assessed by exposing participants to 16 computer-based images of common snack foods spanning caloric content and macronutrient composition (low/high fat sweet, low/high fat savory). After each 5-second snack food exposure, participants

will provide ratings of how much they like the food and how pleasant it would be to eat this food right now. Ratings will be made on visual analog scales anchored by 0 (not at all) to 100 (extremely). The food reward task will be administered at the Baseline (Week -2), Mid.-Tx 1 (Week 1), Mid.-Tx 3 (Week 4), and End of Treatment (Week 8) Visits.

Food Reinforcement (RRVF): The Relative Reinforcing Value of Food will be assessed via a validated behavioral choice task, permitting the evaluation of the preference for food over other alternatives. Participants will be asked to move a computer mouse to hit targets on one of two sides of a split-screen monitor, to earn points toward either food or money. Participants selected the snack food (salty or sweet) that they wanted to consume for the assessment at the Intake Visit. Using a concurrent schedule, participants will be told that they can switch from working on one side of the screen to the other as often as they wish. Adapted from previous research, participants will be instructed to move the computer mouse to have the cursor hit the targets (either a $ or food). Consistent with relative reinforcement paradigms, the reinforcement schedule in the money-earning screen will remain constant at a fixed ratio FR-25 (25 targets achieved to earn a point) while the reinforcement schedule for food will increase (require more effort) with a progressive ratio schedule of PR-25x over 10 trials, such that 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 targets will have to be achieved to earn a point. The computer task will be performed until a participant completes 10 trials and accumulates a total of 10 points from which they will earn either $0.10 for each point (i.e., up to $1.00 paid at the end of the task) or a standardized amount (grams) of their chosen snack food for each point. Food earned is consumed at the end of the task to prevent satiation from influencing responding in subsequent trials. Participants will then begin a 30-minute wait period to standardize session duration and ensure that responding is based on reinforcer preference. At the discretion of the research staff, participants who become frustrated with the length of the RRVF task may bypass the 30-minute wait period. Per convention, the RRVF outcome is defined by a breakpoint (maximum amount of responding) for food vs money across the trials. The Relative Reinforcing Value of Food behavioral choice task will be administered at the Baseline (Week -2), Mid.-Tx 1 (Week 1), Mid.-Tx 3 (Week 4), and End of Treatment (Week 8) Visits.

Alternative Reinforcers (freq, enjoy) (PES): Alternative reinforcers will be measured by the adapted Pleasant Events Schedule, designed to assess reinforcers that occur in the natural environment. The 78 items are rated once in terms of frequency (0 = none to 2 = often) and once in terms of enjoyability (0 = none to 2 = very) over a specified number of days, yielding a *frequency* score, an *enjoyability* or subjective reward score, and the cross product is the reinforcement from the activity. This measure differentiates among smoking and treatment-seeking status, is sensitive to changes in reinforcers across smoking cessation treatment, and has been used to assess reinforcers in substance abusers. Alternative reinforcers (PES) will be measured at the Baseline (Week -2), Mid.-Tx 1 (Week 1), Mid.-Tx 2 (Week 2), Mid.-Tx 3 (Week 4), and End of Treatment (Week 8) Visits.

6.5.5 Microbiome Variables

Participants who are participating in the microbiome sub-study will be instructed to provide stool samples for metagenomic and metabolomic analysis on **Week -2 (Baseline Visit), Week 4 (Mid-Tx 3 Visit)**, and **Week 8 (End of Treatment Visit after completing TN treatment)**. Changes in gut microbiome composition, as determined by metagenomics, will be derived by comparing the relative abundance of bacteria from baseline to weeks 4 and 8. Changes in neuroactive metabolites, as determined by metabolomics, will be derived by comparing levels from baseline to weeks 4 and 8. Although we have hypotheses involving specific metabolites, we chose an untargeted metabolomics approach to enable the identification of other biomarkers of importance.

6.5.6 Outcomes

Weight: Weight (primary outcome) will be measured by a physician's scale (pounds) at the beginning of every in-person visit. Participants will be wearing light clothing without shoes. Pre-cessation weight will be computed as the average of weights at the Intake and Baseline Visits prior to any change in smoking behavior. Weight change from Baseline to the 26-week follow-up will serve as a primary weight outcome variable.

Food Intake (24-Hour Dietary Recalls): Food intake (secondary outcome) will be assessed via 3 telephone-administered, 24-hour dietary recalls during the week after (or near) the Baseline, Mid-Treatment 3 (week 4), End of Treatment (week 8) and the 12-week and 26-week follow-up visits (n=15). A trained member of the research staff will use a multi-pass method with an interactive computerized software program, the ASA24® (Automated Self-Administered 24-hour Recall), to determine total kcal/day (outcome variable). The ASA24® was created by investigators at the NCI. Three recalls are considered optimal for assessing dietary intake, especially when weekend and weekdays are assessed, as we propose to do when possible. If participants are unable to complete a scheduled recall over the telephone or are onsite for a scheduled visit, participants may be asked to complete a 24-hour dietary recall with the research staff in-Center. Comparable accuracy can be achieved when administered in-person and over the telephone. Food recalls are widely used, reliable and valid, assessing kcals/day within 10% of actual dietary intake measured under laboratory observation and by doubly labeled water.

Changes in food intake (kcals) from baseline to week 12 will serve as an outcome for the microbiome sub-study.

Smoking Cessation (CO, Urine Cotinine,and Saliva Cotinine): Smoking abstinence (primary outcome) will be assessed and biochemically verified at EOT (week 8), at 12 and 26 weeks after the target quit date. The primary smoking outcome variable will be 7-day point prevalence abstinence (no smoking, not even a puff, for at least 7 days prior to the assessment) biochemically verified by CO < 5 ppm or Urine Cotinine assessment at a sensitivity of 100 ng/mL at EOT (week 8) and Follow-Up 1 and 2 visits (Week 12 and Week 26). Smoking status will also be biochemically confirmed by saliva cotinine < 15ng/ml at the 12- and 26-week follow-up.

Smoking Cessation will be assessed and biochemically verified at EOT (week 8) and at 12-weeks post TQD as a primary outcome for the microbiome sub-study. A reliable and valid timeline follow-back method is used to assess daily smoking (presence and rate). The smoking outcome variable is 7-day point prevalence abstinence biochemically verified by CO < 5 ppm or Urine Cotinine assessment at a sensitivity of 100 ng/mL at EOT (week 8) and Follow-Up 1 and 2 visits (Week 12 and Week 26). Smoking status will also be biochemically confirmed by saliva cotinine < 15ng/ml at the 12- and 26-week follow-up.

Smoking Rate Timeline Follow Back (TLFB): A reliable and valid timeline follow-back method will be used to assess daily smoking (presence and rate) at every visit post Intake.

fMRI Tasks **Neuroimaging Sub-Study Only*: See section 6.2.12: fMRI Scanning Visits for the complete descriptions of the in-scanner tasks.

Food Cue-Induced Craving. The key outcome for this variable is BOLD signal change for most appetizing foods (versus water, and versus least appetizing foods as an active control). Primary regions of interest for the BOLD fMRI analysis are insula, caudate, OFC and ACC. As a secondary measure, food craving will be assessed prior to and after completion of the scan. The food craving assessment is the 3-item "desire" subscale of the reliable and valid state version of the Food Craving Questionnaire (FCQ-S).

Relative Reinforcing Value of Food (via Food Reward Reinforcement/Choice task). The primary behavioral outcome will be the amount of additional effort subjects are willing to expend in order to obtain food. The primary regions of interest for the BOLD fMRI analysis are VS and vmPFC. Within these regions, the food choices (all 50 trials where food is an option) vs. fixation will be the primary contrast. Selection of this primary contrast will ensure the same stimuli will be analyzed across participants and conditions and maximize statistical power for detecting food-related activation.

Working Memory N-Back Task. Based on our prior work, the primary performance measure will be correct reaction time, and the secondary measure will be number correct; the primary regions of interest for the BOLD analysis will be DLPFC and medial/frontal cingulate gyrus.

6.6 Tissue Specimens

Saliva: If a participant reports not smoking (not even a puff of a cigarette) for at least the 7 days prior to the 12-week and 26-week Follow-up Visits without the aid of NRT, the participant will be asked to provide a 5ml saliva sample used to assess cotinine levels and biochemically verify 7-day point prevalence abstinence.

Urine: A urine sample will be required at the Intake Visit, EOT Visit, Follow-Up 1 and 2 Visits, and both fMRI Scan Visits (neuroimaging sub-study only) for drug (~20-30ml),pregnancy , and cotinine screenings . These samples will be disposed of following the conclusion of the study visit.

Stool (microbiome sub-study only): Stool samples will be provided by participants at Week -2 (Baseline Visit), Week 4 (Mid-Tx 3 Visit), and Week 8 (End of Treatment Visit. See the Microbiome Sub-Study procedures for additional detail.

6.7 Sample Size Determination- Parent (Main) Study

For **Aim 1** we hypothesize that BAS+ vs. SC will have higher smoking abstinence rates and less PCWG at the 26-week follow-up (primary outcomes). Power calculations were all approximated as t-tests or z-tests, and conducted using PASS Software (Power and Sample Size, NCSS Software, Kaysville, UT), Bonferroni corrected for two primary outcomes (p=.025). Proportions of abstinent and smoking subjects will be compared using the log of the odds-ratio, which has a known variance. Informed by smoking cessation counseling plus TN research, we anticipate that the 6-month abstinence rate will be 13% in the SC (control) group and 25% in the BAS+ group (consistent with a persistent effect of BAS+ after the discontinuation of TN). A sample of 213 per group provides 82% power to detect this 12% difference in quit rates at 6 months. This represents a clinically meaningful effect and corresponds to a small effect size (δ=.15). For PCWG, we will include all participants in the analysis, while testing a smoking status x treatment interaction. Informed by epidemiological and clinical studies of PCWG, we will have 80% power to detect a 6 pound (2.72 kg) difference in PCWG between BAS+ and SC, using the interaction term. We expect BAS+ vs. SC will have less post-cessation food intake (secondary outcome) at the 26-week follow-up. We have 80% power to detect a 320 kcal/day difference between the two groups (p = .05). In light of these considerations (i.e., 213 subjects per arm; total 426) and an attrition estimate of 15% across accrual, we will recruit a total of 245 subjects to each arm for a total of 490 subjects (~500).

For **Aim 2**, we hypothesize that BAS+ vs. SC, will increase alternative reinforcers, and reduce food reward, the RRVF, and food cue-induced craving. These changes, in turn, will predict less food intake and PCWG, and greater smoking abstinence at the 26-week follow-up. Using structural equation modeling, we will test the treatment effect on the slope of the mediators versus time, with pre-quit values representing the intercepts. We will examine the proportion of

treatment effect explained, and test with a delta method generated z-score (Type I error of .05). There are four proposed mediating pathways between treatment and outcome, so our Type I error will be corrected to 0.0125. For smoking abstinence, a sample of 426 gives us 80% power to detect small effects (δ=.32) for changes in the proposed mediators, where serial observations are uncorrelated within subject. This also applies to individual path strength. Even a modest within-subject correlation (ρ=0.3) provides 80% power to detect a small treatment effect (δ=0.20). For PCWG, smoking status will be treated as a time-varying covariate, where changes in weight are represented as a slope factor (based on observed repeated measures of weight). With a sample of 426, we will have power to detect a small effect (δ < 0.20), even without adjustment for within-subject correlation.

As an **exploratory aim,** we will test whether females, or participants with greater pre-treatment depression symptoms, or weight concerns, are more likely to experience PCWG and more likely to benefit from BAS+ vs. SC. We will test an interaction effect predicting that the effects of the BAS+ will be more pronounced for females, and for those who have greater depression symptoms, or weight concerns. For smoking abstinence, with a 6-month quit rate difference of 12% (25% BAS+ vs. 13% SC), we approximated power by simulation using a z-test based on the ratio of odds ratio (ORR). If this effect, for example, is completely derived from a larger effect of BAS+ among females (no effect in males), then we will have 80% power to detect an interaction with 213 subjects per treatment arm. This corresponds to an odds ratio of ~4.0 for a BAS+ effect in females and an odds ratio of 1.0 in males. All tests will be performed at a $p < .05$ significance level. We approximated the same interaction for PCWG using the z-score. Restricting the sample to abstinent participants (54 BAS+, 28 SC) with continuous moderators dichotomized at the median, a standard deviation of 3.5 kg, and a standard error of 1.63 for the interaction, we will have 80% power to detect an interaction difference of 4.7 kg (10.4 lbs.).

6.8 Sample Size Determination - Neuroimaging Sub-Study

We plan to test hypotheses at a global 5% Type I error (alpha) for each family of hypotheses, and have chosen a completed sample (N=60) that will achieve at least 80% power using 2-sided tests. Our analysis of power was based on “effect size” (standardized difference). Effect sizes were calculated from our own preliminary data, from published data summaries, and from published estimates of effects (e.g., t statistics, coefficients, p-values) using validated summary methods. We have several hypotheses and more than one outcome measure for some (e.g., different brain regions). We will control error in a family-wise manner, adjusting alpha (Bonferroni Aims 1 and 2, Hochberg step-up procedure for Aim 3) for the number of tests within each hypothesis family. Bonferroni corrections will also be adjusted for the correlations among multiple outcomes.

Aim 1 includes 2 hypotheses (**H1a&b**) regarding treatment effects on neural responses including 5 ROIs responding to food-related tasks. We test at $p<0.02$ because of the high correlation among ROIs within subjects (rho=0.54). Estimates of effect size from our center data (d=0.36) indicate that we have 80% power to detect the effects. Aim 1 also tests hypothesis **H1c** for cessation effects. At the Bonferroni corrected threshold of p=0.0167, we have 80% power to detect small to medium effect sizes (d=0.37).

Aim 2 (**H2a&b**) essentially tests for mediation. The hypothesis that cessation increases food intake will be tested at 0.05, with power for effect sizes ~d=0.32 [9, 58]. For Aim 2b (ROIs predicting food intake), we expect a weaker correlation than H1a (rho=0.4), and our adjusted threshold for significance is $p<0.01$. Expected effect sizes are approximately 0.5, and we expect 96% power to detect the effects.

Aim 3 begins with the model in Aim 2b above, and tests interacting effects of working memory-related BOLD signal and food reinforcement (or food cue reactivity) on food intake. We will reduce the complexity of this analysis by using difference scores (pre minus post) to model a 2-

way interaction between working memory and food reinforcement on food intake. We will only test for brain ROIs found to be sensitive to treatment in Aim 1, the number of tests for interaction is uncertain. We anticipate that these effects will be positively correlated and will use the Hochberg step-up procedure, which is robust to correlations among outcomes in the range we expect. For purposes of power calculation, we assumed a correlation of 0.5, which resulted in a corrected p-value of 0.02 for 5 tests. Keeping the independent variables continuous, we can detect an interaction term (one-sample per subject, standardized) of d=0.36 with 80% power. Literature estimates for similar interactions were 0.65, 0.66, and 0.35. The lowest estimate gives 77% power, and the average (0.55) yields 98% power.

6.9 Sample Size Determination - Microbiome Sub-Study

Hypotheses will be tested at a global 5% Type 1 error rate (alpha) for each family of hypotheses, and we have chosen a completed sample (N=60) that will achieve at least 80% power using 2-sided tests. Our analysis of power is based on “effect size” (standard difference). We will control error in a family-wise manner, adjusting alpha using the Hochberg step up method for the number of tests within each hypothesis family. For **Aim 1,** we examined power using a preliminary longitudinal MANOVA to test for overall significance, and then examined the individual terms by bacteria or metabolite. If we have 12 to 18 taxa in our panel, we have 80% power for the overall test if two taxa make a 0.35-SD shift in abundance in response to smoking cessation. This assumes no within-subject correlation. Imposing a within subject correlation and using the jackknife variance estimate (clustered by subject) increases power. For follow-up testing, we will detect individual effects provided that at least one effect size exceeds ~ 1.3.

Aim 2 examines whether the changes in the gut microbiome are associated with food intake and smoking abstinence at week 12. As with Aim 1, we will begin with the omnibus test using the multivariate pairwise distance measure (within subject) as a predictor. For the smoking outcome, we have 80% power to detect an odds ratio of 2.0, reflecting approximately a 20% difference resulting from a 1-SD change in pre-post distance. We also have 80% power to detect a correlation of 0.38, reflecting a 400 Kcal change in food intake. **Aim 3** begins with the model in Aim 2, above and explores interacting effects of treatment (BAS+ vs SC) on the relationship between gut microbiome changes, food intake and smoking abstinence. We will test an interaction effect predicting that the effects of gut microbiome changes (pre-post distance) on food intake and smoking abstinence will favor BAS+ versus SC. We have 80% power to detect a difference of 0.68 in the correlation of pre-post distance with food intake (0.68 versus 0), and a ratio of odds ratios (ORR) of 3.15, corresponding to a 25 to 30% greater change in abstinence for the BAS+ subjects.

6.10 Statistical Methods - Parent (Main) Study

Prior to the analyses, we will: (a) screen the data for data-entry errors, (b) check for outliers, and (c) assess the extent and type of missing data, and select the most appropriate method for dealing with the missing data (e.g., multiple imputation, all available data). We will create all summary scores needed for analysis, and check that distributional assumptions are met. We will assess for participation biases, attrition differences and treatment adherence (controlled for in primary analyses) across treatment arms. Main effects and moderated effects analyses will be conducted using Stata software (StataCorp, College Station, Texas) and mediation analyses will be evaluated using M*plus* software 7.2.

Aim 1 will evaluate smoking abstinence (point-prevalence, binary outcome) and PCWG (kg weight gain, continuous outcome) in the context of a longitudinal model with categorical and continuous predictor variables. We will use generalized linear models fitted with random effects using Generalized Estimating Equations. We will specify the logistic model for the binary abstinence outcome, and Gaussian weight gain outcome. Models will include treatment assignment, time point, adherence measures, and covariates related to the outcome in

preliminary analyses. Comparisons are all between-subjects. We will test for interaction between treatment and time point using the Wald Chi-square, and estimate a common odds-ratio across time points if they show no evidence of differing across time. The primary hypothesis will be tested using the z-score corresponding to treatment assignment at EOT, and at 12- and 26-weeks post quit, with the primary test of hypothesis 1a at 26-weeks post-TQD. Consistent with ITT analyses, we will measure smoking cessation and weight gain in the full sample at the 26-week follow-up, evaluating a smoking status (days x rate) by treatment interaction for the PCWG analysis (H.1.b.). The **exploratory aim** will evaluate variables that modify the effect of treatment on smoking cessation and PCWG. We will enter interaction terms (e.g., treatment x depression) to the models generated for Aim 1. The interactions will be retained if the z-score is significant ($p<0.05$). We will focus first on EOT, then the 12-week and 26-week follow-up time points, and finally the combined effect across time point.

Aim 2 will involve assessing the direct effect of treatment on smoking cessation and PCWG, and the indirect or mediated effects of treatment on these outcomes through the hypothesized sequential mediating mechanisms using structural equation modeling. Mediation exists when a relationship between treatment and smoking cessation or PCWG is accounted for in full, or in part, by one or a set of intervening variables (e.g., alternative reinforcers, food cue-induced craving, food reward, RRVF). Longitudinal change in the mediators will be represented with a slope factor (based on observed repeated measures of each mediator). We will assess the mediation strength (absent to complete) by estimating the effect proportion mediated ($\beta_{indirect}/\beta_{total}$), and determine the significance of effects with Delta method standard errors, with bootstrapped standard errors for validation. We will employ a weighted least squares estimation technique with robust standard errors, and a mean and variance adjusted chi-square test for the estimation of model parameters. Multiple indicators of model performance will be used, including the χ^2 test, Comparative Fit Index (< .95), Weighted (<.90) and Standardized Root Mean Square Residual (<.80), Root Mean Squared Error of Approximation (< .05), and an examination of standardized model residuals. Bayesian Information Criterion and Scaled Unit Information Prior BIC will be used to compare different models.

6.11 Statistical Methods - Neuroimaging Sub-Study

fMRI preprocessing will use standard algorithms in FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) including distortion correction, slice-time correction, motion correction, spatial smoothing, high-pass filtering (120s) and co-registration to the structural MRI. Quality assessment (QA) procedures will examine global and ROI based temporal signal-to-noise ratio (tSNR), absolute and relative motion, and signal spike count. Following preprocessing, subject-level BOLD timeseries analysis is carried out using the general linear model (GLM) as implemented in FSL, with a canonical double-gamma hemodynamic response function (HRF) for convolution, and linear contrasts to estimate task-specific BOLD responses for each individual session. To enhance sensitivity and reliability, including motion regressors in the model will reduce potential motion artifact. For group-level Image analysis, Subject-level statistical maps will be transformed into a common anatomic space (Montreal Neurological Institute, MNI) for group-level analysis. A priori region of interest (ROI) analyses will focus on specific regions for each task. For the n-back, primary ROIs will be MF/CG, and bilateral DLPFC. For the cue and reinforcement tasks, primary ROIs will be VS and ventromedial PFC; secondary ROIs will include MF/CG, amygdala, OFC, and insula. Percent signal change and activation extent within these ROIs will be used for statistical tests of key hypotheses. In addition, we will use voxel-wise random effects analysis in FSL to test for the effects outside of predicted regions. These voxelwise analyses will be conservatively corrected using $Z>3.09$ and cluster probability (family-wise error) $p<0.001$

Hypothesis Testing. Percent signal change data will be exported for hypothesis testing using Stata (StataCorp, Texas).

Aim 1: Identify effects of BAS+ (vs SC) treatment on food related neural activity and behavior: These comparisons are all between subject, and will be analyzed using mixed models regression (xtmixed, StataCorp, Texas). The model predictors will include a binary indicator for group (BAS+ vs. SC). The hypothesis will be tested using the z-score corresponding to the main effect of group. Covariates can be included in the model to control error (sex, BMI, nicotine dependence, age, weight concerns), although we do not expect to make large gains for adding subject-level controlling variables in a mixed model.

Aim 2: Evaluate the relative contribution of these neural processes to post-cessation caloric intake: Our outcome variable is average daily calories based on food recalls. The first comparison (pre vs. post-treatment) is within-subject, and will be analyzed using mixed models regression with subject specific random effects. As before, we include a binary predictor for condition, a categorical indicator for serial day, and we will test using the z-score corresponding to condition. For testing candidate mediators, we will again model calories using mixed models, entering variables as time-varying (condition varying) predictors, along with subject specific random effects. Finally, we will build the larger model, looking for confounding that represents potential mediation. For this purpose, we will use regression-based multi-equation path models. Path Analysis partitions a directly measured bivariate relationship, for example, between abstinence and calories ingested, into direct and indirect causal paths, to tease apart the important components mediating the response. We will deem that mediation has occurred when a relationship between abstinence and food intake accounted in full, or in part, by one or more of the intervening variables (BOLD signal change or subjective responses in cessation vs. smoking). We will assess the strength of the indirect effects through confidence intervals, evaluate the proportion mediated (i.e., partial or complete mediation), and determine significance with Delta method standard errors. To assess the level of mediation for a pathway, we will estimate the ratio of the indirect effect to the total effect (i.e., effect proportion mediated $\beta indirect/\beta total$).

Aim 3: Test whether cessation-induced working memory deficits moderate the relationship between neural responses to food cues and reinforcement and food intake: This implies a 3-way interaction for a repeated measures analysis (e.g. cessation condition by food reinforcement by working memory on food intake). We will reduce the complexity and eliminate the repeated measures by conducting the analysis using difference scores (pre vs post), and will use multiple linear regression to analyze the differences in food intake. We will model the brain responses to cue reactivity and to food reinforcement separately. The models will contain a term for the difference in BOLD signal, and we will introduce a candidate moderator.

6.12 Statistical Methods - Microbiome Sub-Study

For **Aim 1, H1a**, the abundance of bacterial taxa will be analyzed at a community level using pairwise distance between samples, and visualized with Principal Coordinates Analysis. That pairwise distance will be the basis for the omnibus tests in all three aims. Prior to the testing of individual taxa, we will conduct a longitudinal MANOVA (Multivariate ANOVA) based on the profile of relative abundances of the gut taxa of interest. P-values from multiple testing procedures will be corrected to control for a specified false discovery rate using the Hochberg method. For **H1b**, we will repeat the omnibus MANOVA test as in H1a using the absolute levels of metabolites, and follow with tests on individual metabolites should the overall test prove significant. Smoking behavior (smoking rate) will be included in the statistical models for Aim 1 as a covariate, because not all participants will have maintained smoking abstinence from TQD through EOT. **Aim 2** will evaluate the impact of post-cessation changes (pre-post changes) in the gut microbiome on dietary intake (kcals, continuous outcome) and smoking cessation (point-prevalence, binary outcome). We will use generalized linear models fitted using Generalized Estimating Equations. We will specify the logistic model for the binary abstinence outcome, and

Gaussian dietary intake outcome. We will begin the analysis with the effect of the omnibus measure (pre-post distance). Should that prove significant, we will continue on to specific taxa and metabolites. **Aim 3** will explore whether treatment modifies the effect of gut microbiome changes on dietary intake and smoking cessation. We will enter interaction terms (e.g., treatment x microbiome changes) to the models generated for Aim 2. The interactions will be retained if the z-score is significant ($p<0.05$).

7 Risks / Benefits

7.1 Potential Study Risks

The potential risks to participants, their likelihood and seriousness, and strategies to mitigate risks are described below. Participants can choose, as an alternative, to not enroll in this study. Overall, there is minimal risk for serious adverse reactions as a consequence of enrolling in this study. Adverse reactions/AEs will be collected, assessed, and reported as per the study protocol (see section 8: Safety and Adverse Events), federal law, and University of Pennsylvania regulations.

Transdermal Nicotine (TN): Some individuals who use TN patches experience mild skin irritation at the patch site, such as itching, burning, and tingling, which subsides in a few hours. Mild to moderate skin redness, rash, or swelling may also occur. Additionally, participants will be instructed to inform the research staff if they experience an irregular heartbeat or palpations. Sleep disturbances and vivid dreams may occur, but these sleep-related issues can be resolved by removing the TN patch before going to bed and reapplying a new TN patch in the morning. Other side effects such as, nausea, vomiting, weakness, dizziness, and rapid heartbeat occur rarely and may be related to nicotine overdose. Nicotine overdose is most often caused by continuing to smoke while using the patch. If these reactions occur, and the participant is currently smoking and using the patch (i.e., the participant has relapsed/lapsed but still is wearing the patch), participants will be counseled to reestablish a target quit day and gradually reduce their smoking rate. Participants will not be instructed to discontinue their patches if they have a smoking lapse unless a serious adverse event or experience severe/intolerable side effects. If a participant experiences symptoms of an allergic reaction, such as difficulty breathing or notable rash, they will be instructed to cease use of TN and consult with the research staff and Study Physician immediately.

Other nicotine patch risks can include risks to children and pets if TN patches are not stored or disposed of properly. Unused and used patches have enough nicotine to poison children and pets. Participants will be instructed to keep patches out of the reach of children and pets. Participants will be instructed to fold the sticky ends together when disposing of used patches. Participants will be informed that if a child or pet swallows a nicotine patch, they seek professional help or contact a Poison Control Center immediately.

The overall risk of adverse responses to TN will be minimized by only admitting subjects to the study if they do not have preexisting conditions that significantly increase the risk for adverse responses (e.g., heart disease, uncontrolled high blood pressure, allergy to adhesives, poorly controlled diabetes, etc.) per the product labeling. In addition, no TN will be distributed to a participant without the documented approval (via email) of the Study Physician post Intake. The research staff, in conjunction with the Study Physician and Principal Investigator, will monitor for anticipated side effects of TN per the SEC form and other AE assessment measures (see section 8.2.1: AE Collection Methods) at every in-person visit over the treatment period. Participants will be provided with an information sheet describing the proper use of TN, the possible adverse reactions of TN, and the methods for minimizing the possibility of adverse reactions. Participants will be specifically instructed to contact the study staff or the Study

Physician if they 1) experience severe or persistent skin reactions (e.g., skin redness, itching, or swelling) caused by the patch that last more than 4 days, or a generalized skin reaction (e.g., hives or rash); 2) notable irregular heartbeat or palpitations (e.g. notable hard or fast beats); 3) persistent nausea, vomiting, dizziness, weakness, or rapid heartbeat (symptoms of nicotine overdose); or 4) symptoms of an allergic reaction such as difficulty breathing or notable rash. Participants should not stop using the patch without discussing their symptoms with the Study Physician and study staff unless they experience severe or intolerable side effects. The Study Physician is knowledgeable of side effects related to TN treatment and is qualified to manage possible side effects. Any severe adverse reactions or significant side effects of TN as identified in section 8.2: Safety and Adverse Events will be medically evaluated by the Study Physician.

Reproductive Risks: Because TN safety for an unborn baby is unknown, participants will be told that they should not become pregnant while in this study. Women in the study should not breast-feed a baby. If a woman is of childbearing potential, she must agree to use an adequate form of contraception or abstain from sexual intercourse for the duration and for at least one month after the end of the trial. If a woman is pregnant or breast-feeding, she should not participate in this study. If she becomes pregnant during the study, she will be asked to notify staff immediately and will be removed from the study. Women will be asked to self-administer a pregnancy test during the Intake Visit (prior to the TN treatment period).

Withdrawal Syndrome: Many individuals who quit smoking exhibit a pattern of symptoms related to withdrawal from tobacco use. These symptoms include: anger, irritability, frustration, anxiousness or nervousness, depressed mood or sadness, cravings for nicotine, difficulty concentrating, appetite change and weight gain, insomnia or other sleep problems, restlessness, impatience, constipation, dizziness, coughing, nightmares, nausea, sore throat, headache, muscular pain, or fatigue. Eliminating the risk for these would not be possible, although in most cases these events are short-lived and have low intensity, lasting for 1-2 weeks. The study personnel will be trained to recognize these symptoms and educate the participants about them (e.g., duration and methods for reducing them).

Food Allergies: Participants will be provided with nutritional shakes and potato chips or milk chocolate and as part of their participation in this research trial. The risk of a participant experiencing an allergic reaction to any of the ingredients in these commercially available food products will be mitigated by prospectively excluding those with the applicable food allergies. In addition, the research staff will reconfirm that the participant doesn't have any of the applicable food allergies prior to distribution of the aforementioned food products at the Baseline Visit.

Psychological Distress: Participants may experience emotional distress during smoking cessation counseling and assessments from discussing feelings and attitudes about smoking or from learning about the risks from smoking. These events happen very rarely and in almost all cases are short-lived and of low intensity, lasting for 1-2 weeks. Study personnel will be alerted to expect this from a small number of subjects and will be trained to make referrals for mental health services as needed. Personnel will be trained to query for adverse emotional reactions during counseling and assessments and will be trained to deal with such reactions and to provide additional referrals if needed.

Urine and Saliva Sample Collection: Participants may experience mild discomfort from providing urine or saliva samples. All samples will be collected by trained member of the research staff.

Email Communications: In this research study participants may prefer to receive appointment reminders via email or submit questions related participation via email. Email is not a secure means of communication. Email messages travel across the Internet passing through multiple

computers before reaching their final destination. It is not possible to know whether an email a participant sends will be viewed along the way. Additionally, if sent messages are not deleted, an email provider may have an archive of everything that is sent. If someone gets access to an email account (for example, a participant's family member), they could see archived messages. There are many other ways in which emails are not secure—these are only selected examples. To manage this risk the informed consent form will include specific language to educate research participants on the privacy risks involved in email communications. Participants will also be explicitly instructed to only use email communications for routine matters and never for personal or confidential messages or questions.

fMRI: The known MRI-related risks associated with this study are minimal. All sequences and RF coils will be approved by the Center for Magnetic Resonance Imaging and Spectroscopy (CAMRIS) prior to utilization. Because of the strong magnetic field, people with pacemakers, certain metallic implants, or metal in the eye cannot participate in this study. These exclusions will be reviewed carefully with the study staff prior to scanning. Although the Nicoderm® CQ® package insert states that patches should be removed before undergoing any MRI procedures – "for the opaque Nicoderm® CQ® patch only," we will err on the side of caution and instruct participants in the neuroimaging sub-study to remove their 7mg Nicoderm® CQ® - Clear patch before entering the MRI environment during their Scan 2 Visit only (no patch worn during the pre-treatment scan). Participants will be provided with a loose, sealed 7mg (NicoDerm® CQ® - Clear) patch to reapply after exiting the MRI environment.

The greatest risk with MRI is a metallic object flying through the air toward the magnet and hitting the participant. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. No metal objects are allowed in the magnet room at any time. In addition, once the participant is in the magnet, the door to the room will be closed so that no one inadvertently walks into the magnetic field. Although there are no known risks of MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no direct benefit from participating in this protocol for a pregnant woman, women of child bearing potential will be supplied with a simple, CLIA-waived urine pregnancy screen to self-administer at each scan visit, and will be told that pregnant women may not participate in the study. They will then be instructed to administer the pregnancy test independently and will inform study staff if they would like to continue participation after they have administered the pregnancy screen. Participants will be informed that there is no penalty for discontinuing participation at this point in the visit and that they will still receive travel reimbursement for the visit.

There is no known health risk associated with exposure to magnetic fields during an MRI. There are minimal risks from the loud noise associated with the MRI scanner and from the discomfort of lying on a hard surface. Participants will be provided with protective earplugs as necessary and every attempt will be made to ensure comfort with blankets, etc. during the scan.

The levels of energy used to make magnetic resonance measurements are far less than are used in a single X-ray, and many patients have been safely studied using magnetic resonance techniques. However, some people become uncomfortable or claustrophobic while inside the magnet. If participants become uncomfortable, they may withdraw immediately from the fMRI portion of the study. During some of the MRI scans, participants have occasionally reported "tingling" or "twitching" sensations in their arms or legs, especially when their hands are clasped together. To prevent this, all participants will be instructed to keep arms and legs apart.

The imaging component of this study is part of a research protocol, and is not intended to provide a comprehensive clinical fMRI examination of the brain. In the event that a significant

brain abnormality is observed while processing subject brain images for the research study, the images will be reviewed by the radiologist affiliated with this protocol in conjunction with the Study Physician. There will be no charge to the subject for this examination of their images. A report will be filed in the subject's chart at the Center. The subject will be contacted and Center staff will arrange for the radiologist's report and structural images to be sent to the subject and/or their physician. Subjects will also be offered a consult with the Study Physician and/or radiologist if appropriate. These possible finding(s) may or may not be significant and may lead to anxiety about the subject's condition and to further work-up by the subject's physician.

Risks associated with stool samples (microbiome sub-study only):
Collecting stool may contain germs that spread disease. Participants will be reminded to carefully wash their hands and use the provided gloves to avoid spreading infection. Some people may feel uncomfortable or embarrassed using the stool sample collection kit. There should be no pain while collecting the stool sample. However, if a participant is constipated, straining to pass stool may be painful. We will discuss this risk with the participant during the informed consent presentation to ensure that the individual subject is comfortable with the process.

Risks associated with genetic testing (microbiome sub-study only):
This research includes genetic testing. We will sequence all the genomes present in each stool sample (both human and microbial) via a technique called shotgun metagenomics. Part of the analysis process involves assembling the microbial reads into contigs. We do not assemble contigs from the human data. Even without a name or other identifiers, a participant's genetic information is unique to them. The researchers believe the chance that someone will identify a participant is very small, but the risk may change in the future as people come up with new ways of tracing information. We will not complete genetic testing of inherited traits for research purposes.

Confidentiality and Loss of Privacy: See section 9.7.1 and 9.7.2 for methods in which Confidentiality and Subject Privacy/Protected Health Information will be secured and maintained.

7.2 Potential Study Benefits

Participants who enroll in this trial will benefit from the knowledge that they are contributing in an important way to potentially furthering scientific knowledge concerning ways to improve treatment for smokers, and prevent PCWG. Weight gain can precipitate smoking relapse and contribute to other health issues. All eligible smokers will receive TN and behavioral counseling and may quit smoking and learn skills to stay quit as a result of participating.

7.3 Risk/Benefit Assessment

There is minimal risk for serious adverse events by enrolling in this research study. The treatments and procedures used in this study have been shown to be relatively safe. Numerous clinical trials have demonstrated the safety and efficacy of transdermal nicotine and behavioral counseling for nicotine dependence. Research staff will monitor subjects closely during their participation. Thus, the risk to benefit ratio for this project is perceived to be low and justifies its implementation.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others:
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event:
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study (regardless if TN related). Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event:
Adverse events are classified as serious or non-serious. A ***serious adverse event*** (SAE) is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period:
The study period during which AEs/SAEs will be reported is from the initiation of any study procedures until the end of the study. Any event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in the study after the final time point will be assessed and reported as appropriate.

Preexisting Condition:
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Post-study Adverse Event:

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery:
Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Collection and Recording of Adverse Events

8.2.1 AE Collection Methods

All AEs and SAEs occurring during the study period will be captured through the methods described below:

1. SEC: Participants will complete an SEC at all in-person visits during the treatment period (including Pre-Quit as a baseline measure) after the Baseline Visit. The SEC will assess the severity of side effects that may be TN related and experienced by participants in the study (See section 7.1: Potential Study Risks; Transdermal Nicotine). These reports will be formally documented as **anticipated (expected)** AEs whether or not the event(s) is ultimately deemed related or not related to the use of TN in this study. The reporting period for each assessment will inquire about any side effects experienced since the last in-person visit. Items will be rated by participants utilizing the following severity scale: 0 (None=No Concerns), 1 (Mild=Side effect does not interfere with usual daily activities), 2 (Moderate= Side effect does interfere with some activities), and 3 (Severe=No normal activities are possible). Any side effect rated **severe or moderate** will require additional follow up per the internal reporting procedures outlined below in section 8.2.2. **Mild skin redness, skin rash, skin swelling, irregular heartbeat, or heart palpitations** also require additional follow up per the internal reporting procedures outlined below in section 8.2.2. Other side effects reported as mild**,** but not identified above, do not require additional follow up from the research staff or communication with the Project Manager, Study Physician, and Principal Investigator. However, these mild reports are maintained within the data management system and are available via aggregate report.
2. Open-Ended AE Form: Participants will be asked an open-ended question about any symptom or medical event that may be related to their study participation not included on the SEC form at all in-person visits after the Baseline Visit. These events will be

documented as **unanticipated (unexpected)** AEs unless they are otherwise outlined in the protocol or consent (i.e. related to withdrawal, assessments, etc.). The reporting period for each assessment will inquire about any event(s) or symptom(s) experienced since the last in-person visit. If a participant reports a symptom or medical event, they will be asked to rate the severity of the event utilizing the following severity scale: 0 (None=No Concerns), 1 (Mild=Issue does not interfere with usual daily activities), 2 (Moderate=Issue does interfere with some activities), and 3 (Severe=No normal activities are possible). Any report on the Open-Ended AE Form will require additional follow up per the AE documentation and internal reporting procedures outlined below in section 8.2.2.

3. Spontaneous Assessment: Once enrolled, participants will be instructed to inform the research team about any notable symptom or medical event/concern throughout their participation in the study. A participant may also request the Study Physician be consulted about any reported medical event or concern of any severity at any time throughout their participation. At the Pre-Quit Visit (Week -1), participants will receive an emergency medical card (EMC) with the Study Physician's direct contact information should a medical issue or concern that they believe may be related to the study procedures require immediate attention.

   An "AE Note" template will be available to the research staff to collect supporting AE information and will function as the source document. However, research staff may collect AE information on any source document available to them and transfer the relevant information to a formal AE Note at a later time. Any notable AE reported spontaneously will require additional follow up per the AE documentation and internal reporting procedures outlined below in section 8.2.2.

8.2.2 AE/SAE Documentation and Internal Reporting Procedures

AE/SAE Documentation: As noted above in section 8.2.1, research staff are trained to collect follow-up information about any **severe**, **moderate, or select mild side effects** reported on the SEC Form, **any medical event(s) reported on the Open-Ended AE Form,** or **any notable spontaneously reported medical event or concern**. At a minimum, follow-up information will include AE/SAE onset/resolution, description of event/course, severity, action taken, outcome, and possible relation to TN treatment (if applicable).

Information surrounding AEs and SAEs will be initially recorded on the appropriate source document such as the SEC Form, Open-Ended AE Form, an "AE Note" or SAE Form, and/or any document in which the AE/SAE information was originally recorded. All applicable AEs and SAEs will then be documented on a cumulative AE and SAE log maintained within the regulatory binder.

Completed documentation of applicable AEs will include the following information:

- Protocol Title and IRB#
- Subject Identifier
- Event Title
- Date Site Notified
- Event Start Date and Time
- Event Stop Date and Time
- Description of Event/Course (including sequelae)
- Severity:
  - None = No concerns
  - Mild = Side effect or issue does not interfere with usual daily activities

  - Moderate = Side effect or issue interferes with some activities
  - Severe = No normal activities are possible
- Relatedness to the study procedures (PI and/or Study Physician):
  - Unrelated = Definitely not related
  - Unlikely = Doubtfully related
  - Possibly = May be related
  - Probably = Likely related
  - Definitely = Related
- Expectedness per protocol and/or consent
  - Expected/Anticipated
  - Unexpected/Unanticipated
- Action(s) taken (if appropriate)
- Outcome (if appropriate)

Documentation of SAEs will include the following information on a standardized SAE Form:

- Protocol name and number
- Subject identifiers
- Demographic data
- TN Lot number, expiration date, and other descriptive information (if appropriate)
- Date Site Notified
- Date and time of SAE onset
- Date and time of SAE resolution, if available
- Course/Description of Event (including sequelae)
- Action Taken
- Outcome
- Follow-up plan
- Serious Status (What makes the event an SAE)
- Severity of the event
  - None = No concerns
  - Mild = Side effect or issue does not interfere with usual daily activities
  - Moderate = Side effect or issue interferes with some activities
  - Severe = No normal activities are possible
- Relatedness to the study procedures (PI and/or Study Physician):
  - Unrelated = Definitely not related
  - Unlikely = Doubtfully related
  - Possibly = May be related
  - Probably = Likely related
  - Definitely = Related
- Clinical assessment of subject conducted at time of SAE (if appropriate)
- Results of any laboratory tests and/or diagnostic procedures (if appropriate)
- Autopsy findings (if appropriate)
- Concomitant medications and therapies (excluding treatment of event)
- Relevant Medical History (if appropriate)

Internal Reporting Procedures: All relevant follow-up information outlined above (see AE/SAE documentation) concerning applicable AEs, including all information regarding the occurrence of concurrent smoking and TN (if applicable) and previously reported event(s) and/or side effects, will be reported to the Study Coordinator (or other senior personnel), Principal Investigator, and

Study Physician to determine a course of action (e.g. continue to monitor, reduce medication dose, stop medication), relatedness (causality) to the study, and expectedness (if not already established). This consult will be documented via email. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not (or unlikely) to be the cause.

SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome unless it has been determined that the study treatment or participation is not the cause. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately per this protocol.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

The reporting requirements of applicable SAEs and/or Unanticipated Problems including reportable AEs (see section 8.1 for definitions) to external entities are detailed in the following sub-sections:

8.3.1 Investigator reporting: notifying NCI and FDA

In adherence with NCI reporting requirements and GCP standards regardless of IND status, the procedures for reporting SAEs (detailed below) suspected/associated with TN to the FDA are as follows:

1. An SAE that is drug-related (reasonable possibility or related) and unexpected will be reported to the FDA within 15 calendar days of discovery of event.
2. An SAE that is drug-related (reasonable possibility or related), unexpected, and life-threatening or fatal will be reported to the FDA within 7 calendar days of discovery of the event.
3. The FDA will be notified of applicable SAE reports via the MedWatch form/system (FDA Form 3500).

The NCI program officer assigned to this trial will be notified of any actions taken by IRB with regard to data safety monitoring.

8.3.2 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems <u>within 10 working days from the time the investigator becomes aware of the event:</u>

- Any AE (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the Principal Investigator and Study Physician is:

  <u>Unexpected</u> (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

Related to the research procedures (According to the Penn IRB standard operating procedures [SOPs], an event is "related to the research procedures" if the event is deemed **probably or definitely related** to the procedures.")

Reporting Process:
Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths (more rapid reporting requirements):
Deaths that occur during the course of a research study and that are:

- Unexpected; AND
- Related to the research study; AND
- When other participants are believed to be at an increased risk of harm

Must be reported to the IRB within 3 days from the time the investigator becomes aware of the death.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.4 Medical Monitoring

It is the responsibility of the Principal Investigator and Study Physician to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of AEs/SAEs as noted above, as well as adherence to the study data and safety monitoring plan outlined in sections 8 and 10.

9 Data Management

The CIRNA Data Management Team has developed a data management system (DMS) that will facilitate the operational facets of this study, including determination of entry eligibility, production of lists of subjects for telephone contacts for scheduling, and data entry. The DMS uses the relational database product Microsoft Access as the primary software platform for data entry and validation, storage, retrieval, modification, and security. The DMS ensures data integrity through range and validity checks during the data entry process. Daily backups are performed to protect data against accidental destruction or corruption.

9.1 Data Management System Development

The CIRNA Data Manager will work closely with the trial investigators to develop an understanding of the data collection, storage, and quality assessment needs for the trial. This includes the design and development of the trial data collection forms and any additional administrative CRFs, to ensure that standardized, uniform data collection and data management procedures are implemented and sustained throughout the trial. The data collection forms will serve as templates for designing the data entry screens. The Data Manager will work closely with trial investigators and senior personnel to design, develop, and test an appropriate database structure to support the requirements of the DMS and to promote data security and integrity. Electronic audit trails of changes to database contents are incorporated into the design and will capture and record those changes automatically. In addition to the trial database where actual results will be maintained, a development database will be created. The development database is a working environment that facilitates the development, testing, troubleshooting, enhancement, and training for the DMS without adversely affecting the integrity of the collected project data.

Prior to deployment and use by the research staff, the database and DMS will be subjected to extensive functional testing. This testing is conducted according to a written test plan and is intended to verify the proper functioning of all components of the DMS. Any components that do not function as they were intended will be identified and evaluated by the development team to determine appropriate corrective action. Testing will also include an evaluation by user representatives for adherence to the requirements established by the intended users for the DMS. Successful completion of these user acceptance tests will mark the end of development and predicate the deployment of the DMS for use in storing and managing active trial data. Any modifications made to the DMS will be conducted in accordance with change control procedures.

9.2 Data Security

All research data for the trial will be stored in an electronic Access database that is managed by the Data Manger. The database will be hosted on a secure computing server and will be restricted to only those individuals who are authorized to work on the trial. Individual user accounts with passwords will be used to restrict access to the database. Specific privilege assignments within the database will also be employed to limit the types of functions that authorized users can perform to those functions that are appropriate for their role in the trial.

Additional measures to prevent unauthorized external access to the database environment will be employed using network firewall technologies. The Data Manager will maintain the database in an appropriate manner for the retention period required by regulation. Database administration includes user account maintenance, database security, performance monitoring, and database change management.

Storage and archiving for imaging data will be stored on The Center for Functional Neuroimaging (CfN) secure computer cluster.

9.3 Data Processing

The data entry screens will resemble the data collection forms as closely as possible to allow visual referencing during data entry. This data entry module will be configured for single data entry. Participant data will be collected by research staff, recorded on study-specific CRFs, and scanned in or entered directly into the appropriate DMS module. Data entry checks will be included in the entry screen designs where appropriate to limit the opportunity for erroneous entries due to mistyping. Such data entry checks would include value range comparisons, valid data type checks, required value checks, and/or skip pattern enforcement. Following telephone eligibility screening, research staff will perform subject registration. Following the Intake Visit, research staff will randomize eligible subjects. The randomization module will allow the research staff to randomize subjects into one of the two trial arms. At the randomization attempt, the DMS will check the eligibility data to confirm that randomization is valid. A randomization assignment will then be provided.

9.4 Data Quality Assurance

A data quality module will be developed to assess data entered into the database in relation to a set of rules that describe expectations for those data items. This set of data validation rules will be defined by the data manager, working closely with trial investigators, to identify data items that may have been collected incorrectly or entered into the database inaccurately. The module will run automatically to inspect all newly entered or modified data. The research staff will review the results of the data validation and take any required corrective action for invalid data. Queries will be recorded and tracked in the data quality module. Corrections identified for individual data items will be managed by the research staff. All changes made will be recorded in an electronic audit trail and documented using change control procedures.

Monitoring of trial progress will be accomplished, in part, through the use of standard reports. The Data Manager will program a set of standard enrollment, tracking, quality review, and safety monitoring reports. Data audits will occur after the first few participants are enrolled and periodically during the trial to detect errors in data entry. Eligible participants will have 100% of their source document information compared with the data entered in the database. Any errors will be investigated, resolved, and a plan will be implemented to prevent further errors should concerning patterns emerge.

9.5 Subject and Specimen Tracking

The Data Manager will develop a module to assist research staff in recruitment and retention tracking for trial subjects. This module will accept and store contact information for potential subjects and will include data items to indicate the completion status of significant events. The tracking module will include information about contact and visit schedules to assist in preparing communications to potential subjects and trial participants concerning scheduled events. The module will also allow for incentive-related inventory management. When obtaining saliva specimens, the research staff will complete a specimen registration CRF and scan/enter the data into the DMS. A unique specimen identifier will be assigned and recorded on the CRF. Labeled specimens and applicable information will be transferred to the lab for analysis as required for analysis.

Stool specimens (microbiome sub-study) delivered to the CHOP Microbiome Center and the Penn Metabolomics Core will be labeled by study ID only. Samples will be processed, analyzed, stored, and logged at the CHOP Microbiome Center and Penn Metabolomics Core. Additionally, stool sample tracking and collection information (i.e., sample status, date returned, date transported, etc.) will be tracked via the study DMS.

9.6 Retaining Data and Biospecimens for Future Research Use

Permission will be sought from participants that elect to enroll in microbiome sub-study to retain their information and stool samples for possible use in future research. Providing permission is optional and the participant's choice will be documented and maintained on both the combined Informed Consent and HIPAA Form and the study DMS. Participants will not directly benefit from future research with their information and stool samples, but they will be informed that the information and stool samples that they provide could be useful to future researchers by improving the understanding of health and disease, improving health care, making safer or more effective medical therapies, and developing new scientific knowledge. There are no plans to inform participants about any of the specific research that will be done. Further, we will not provide participants with any results from these future studies. It is possible that participants may have chosen not to participate in these future research studies, had they been approached.

Although participant data and stool samples collected in this study will be labeled and stored with a study identification number only, there is a possibility that a study identification number and personal identifiers could be linked. See the Data Handling and Record Keeping section for methods and descriptions about how Confidentiality and Participant Privacy/Protected Health Information will be secured and maintained.

Participants may change their mind and withdraw their permission for the future use of their information and stool samples at any time by contacting the Principal Investigator and informing them that they no longer want their information and stool samples to be retained for use in future research. Although most uses of biospecimens or information do not lead to commercial products or to profit for anyone, participants will be informed that it is possible that their stool samples may be used for commercial profit and that there are no plans to tell them, or to pay them, or to give any compensation to their family. Additionally, participants will be informed that individual research results obtained as part of future research will not be shared with them.

Participants' information and stool samples will be stored for future research purposes only. Participants' information and stool samples may be retained and used for future research for an indefinite amount of time. Future researchers may receive information that could identify participants. This can be done without seeking participants' consent in the future, as permitted by law. The future use of participant information and samples only applies to the information and stool samples collected during this study.

9.7 Data Handling and Record Keeping

9.7.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI. Note in the event that a subject revokes authorization to collect or use PHI, the investigator, by

regulation, retains the ability to use all information collected prior to the revocation of subject authorization.

Confidentially of study data will be maintained in the following manner:

- Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study.
- Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords.
- Prior to access to any study-related information, personnel will be required to review and sign statements agreeing to protect the security and confidentiality of identifiable information.
- Whenever feasible, identifiers will be removed from study-related information.
- Precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys.
- Audio recordings will be reviewed by the Principal Investigator for training purposes and then deleted to eliminate audible identification of subjects.

Since self-report and biological data will be collected and stored as part of this study, it is possible that subject privacy or confidentiality can be threatened. To address this concern, the data management system has set up several safeguards to prevent unauthorized access to participant data. In the subject map table, an automatically generated index number is assigned to a subject's study identification number. A linked subject identification table is created to store subject name, address, and telephone contact information. This table uses the automatically generated index number rather than the study identification number. The master subject map and subject identification information are maintained in separate locations. Using this method, no identifying subject information is directly linked to bio-samples or results. Any publication of data will not identify participants by name or with an identifier that could be used to reveal identity.

All biological samples will be labeled with study ID only. All subject data that can be linked to the study ID will be stored in the secure data management system, which has limited, password-required access. The aforementioned precautions and procedures will be applied to protecting subject privacy and the protected health information detailed in Section 9.7.2 below.

9.7.2 Subject Privacy/Protected Health Information

The following protected health information (PHI) may be collected as part of this study:

1. Name
2. Street address, city, county, zip code
3. All elements of dates (except year) for dates directly related to an individual and all ages over 89
4. Date of birth
5. Social Security Number
6. Telephone number, email address
7. Any other unique identifying number, characteristic, or code
8. Results from all questionnaires, tests, and procedures

Potential participants will be contacted over the phone after responding to recruitment efforts or having agreed to be contacted for future studies. Only individuals who have responded to recruitment efforts or who have agreed to be contacted regarding research studies at our Center will be contacted. If an individual cannot be reached immediately, staff members will identify themselves only as calling from the University of Pennsylvania; no mention will be made of the inquiry regarding study participation. Participants will undergo an initial telephone screening where preliminary eligibility for the research study will be determined. Only if a participant is

initially eligible, will they be asked to attend an in-person Intake Visit to confirm eligibility. All data collected over the phone and during in-person visits will be collected by research staff that have completed the CITI-Protection of Human Subjects Research Training as well as HIPAA Compliance Training. Once enrolled, information will never be recorded with identifiers other than study ID. A separate list of names with ID numbers will be accessible only by authorized personnel. All records will be kept in locked filing cabinets to maintain confidentiality. All analyses will be conducted on de-identified data.

Data will be accessible only to the Study Investigators, Study Physician and Study Radiologist, study staff, applicable Center staff, UPenn IRB, Office of Clinical Research, CAMRIS, authorized UPENN and CHOP staff (e.g. accounting and billing matters, provide treatment, oversee MRI scans [MRI Technicians], stool analysis, etc.), PennCHOP Microbiome Program (CHOP Microbiome Center), Penn Metabolomics Core, National Cancer Institute, and the FDA.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify a subject directly. At most, the website will include a summary of the results. Subjects may search this website at any time.

10 Data and Safety Monitoring

10.1 Research Roles

During the course of the study, data and safety monitoring will be performed on an ongoing basis by the Principal Investigator, Study Physician, research staff, and the IRB. The research staff are responsible for collecting and recording all clinical data. This includes ensuring that all source documents exist for the data on the case report forms (CRFs), ensuring all fields are completed appropriately, and all error corrections are done according to GCPs. Any inconsistencies/deviations will be documented and addressed as appropriate. The research staff will perform regular chart reviews to verify data integrity. The Study Coordinator (or senior personnel) and Principal Investigator will maintain the study regulatory binder/essential documents per GCP. The Study Physician will be available to review medical issues related to participation for each participant on an ongoing basis as outlined in this protocol. Research staff will meet and communicate on a regular basis to reconcile data queries and safety concerns. The IRB will review the trial on an on-going basis per institutional and federal regulations until the study is formally closed-out.

CAMRIS will be responsible for the regulatory oversight of all MRI related activities within this protocol including initial review and approval of the neuroimaging sub-study.

10.2 Staff Training

Staff training will consist of an initial explanation and review of the protocol, informed consent form, CRFs and laboratory tasks, sample collection protocols, data management system, adverse event collection and reporting, and all study-specific SOPs. In addition, during a standardized training period, the duties of each staff member will be clearly outlined and all applicable regulations will be reviewed. The Principal Investigator, Dr. Audrain-McGovern, will oversee the behavioral activation intervention and smoking cessation counseling training, as well as the transdermal nicotine procedures. Training interactions will be documented in a training log, which will be maintained within the regulatory binder. Senior personnel will supervise junior staff and provide re-training as needed.

Dr. Loughead and senior staff will oversee the development of protocols for activities pertaining to the neuroimaging sub-study and training of staff in these protocols. Dr. Loughead and/or the Project Manager will also be responsible for the development of procedures pertaining to all

neuroimaging study visits and implementing and monitoring ongoing staff training procedures accordingly.

All personnel working on this project will complete required training in the protection of human subjects and the protection of personal identifiable information (i.e. HIPAA) before interacting with study data or research participants. All human subject and privacy protections certifications will be maintained in the regulatory binder.

10.3 Monitoring Activities

10.3.1 AE/SAE Monitoring

Monitoring and management of AEs/SAEs will be conducted in real-time by the Principal Investigator, Study Physician, and the research team at regular time points as per the methods and procedures detailed in section 8: Safety and Adverse Events.

10.3.2 Initial Assessment (Intake) Monitoring

The study staff will conduct a manual review of source documents and CRFs for all subjects determined to be eligible at telephone screen and again prior to the Intake Visit. Eligibility data will be reviewed in real-time at the Intake Visit by the research staff. In addition, The Study Coordinator (or senior personnel) will verify that all data have been collected and, when applicable, meet the eligibility criteria on a "Final Eligibility Checklist." The Final Eligibility Checklist will be signed and dated by the Study Coordinator (or senior personnel) to formally document review. In addition to confirming eligibility, a brief, internal report describing the findings will be compiled and distributed to study staff (if applicable). If the Study Coordinator (or senior personnel) notes a pattern of improper data collection or deviations, additional trainings will occur.

After the Intake Visit, but prior to TN distribution at Pre-Quit, the Study Coordinator (or senior personnel) will inform the Study Physician that applicable participants have met all the eligibility criteria (i.e. did not present with any exclusions) including blood pressure, medical history, and concomitant medications via email for formal approval to dispense TN. The Study Physician's approval via email will be maintained in the participant's study chart, as well as on file in an electronic format. The Study Physician will be available to respond to any additional eligibility queries as well.

10.3.3 Protocol Monitoring

Protocol monitoring includes a survey of those activities that are associated with protocol adherence such as identifying, reporting, and rectifying protocols deviations, reviewing for violations of inclusion/exclusion criteria, and ensuring the adherence to study-specific SOPs, GCP, and other federal and institutional regulations. Protocol monitoring will be performed on an ongoing basis through the following methods:

1. Checklists will be utilized at all time points to ensure all data is collected per protocol and procedures are followed as appropriate.
2. A Final Eligibility Checklist will be completed after the Intake Visit for all participants who enroll (i.e. sign consent) in the study. The Final Eligibility Checklist will serve as final confirmation of eligibility status prior to seeking approval for TN distribution from the Study Physician. Additionally, a neuroimaging sub-study Final Eligibility Checklist will be completed in order to confirm sub-study eligibility for those participants who met initial sub-study eligibility at Phone Screen and elected to participate in the sub-study at Intake.
3. An internal chart review procedure will be completed for ~25% of randomly selected eligible subjects. The chart review procedure is a thorough review of all source documentation to ensure the integrity of the data, all study paperwork is present, all fields are completed per GCP, and all study-specific SOPs have been followed appropriately.

10.3.4 Database Auditing

As outlined in section 9: Data Management, the study DMS will be equipped with internal validation checks to ensure data is entered within reasonable ranges. Error messages will be displayed in real-time if data appears inaccurate. Staff will have to respond to these error messages before data can be saved. In addition, The Study Coordinator (or senior personnel) will perform regular milestone quality assurance checks.

10.3.5 Data Security

As outlined in section 9: Data Management, study data will be secured through controlled user access and accessible to authorized personnel only. Source documents will be secured in locked filing cabinets.

10.4 Frequency of Data and Safety Monitoring

Data will be reviewed internally on a regular basis. Specifically:

1. At data capture, the research staff will review data for completeness and integrity.
2. At data entry, the DMS will include multiple internal validity checks which will prompt the staff if an entry was made that is out of range or in an unacceptable format.
3. Eligibility data will be reviewed in real-time at the Intake Visit. In addition, the Study Coordinator (or senior personnel) will review and verify that all data have been collected and, when applicable, meets the eligibility criteria on a “Final Eligibility Checklist.”
4. Between the Intake Visit and TN distribution (Pre-Quit), the Study Physician will receive confirmation from the Study Coordinator (or senior personnel) that a participant has met all the eligibility criteria via email. Any additional eligibility queries will be addressed at this time as well. No TN will be distributed without documented approval of the Study Physician via email.
5. On a regular basis, the project staff will review data through an internal chart review procedure supported by the DMS. A random subset of eligible participants (~25%) will be reviewed.
6. All CRFs for eligible subjects are 100% source-data verified through an internal data management system (Data Entry/Quality Assurance) on an ongoing basis.
7. The study statistician will review data prior to analysis to ensure integrity and validity.

10.5 Auditing and Inspecting

The Principal Investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The Principal Investigator will ensure the capability for inspections of applicable study-related facilities. Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning

the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

11.1 Informed Consent

A fully trained staff member will obtain informed consent using the combined consent and HIPAA form approved by the IRB (UPENN). The consent process will take place prior to the initiation of any study procedures. **If the PI deems it is safe for the participant and research staff, based upon federal and University COVID-19 guidance,** then the consent process will occur in person at the CIRNA and will involve a discussion of the study requirements and procedures. The combined consent and HIPAA form will be read verbatim to participants. Participants will have an opportunity to ask any questions and/or express concerns. To limit the number of people at the research center and the duration of Intake visits for the safety of the research staff and participants, the combined consent and HIPAA form will be completed virtually via REDCap. Participants will be required to reach the entire consent in order to participate in the research study. Participants will have the opportunity to indicate if they have any questions and a trained research staff member will contact them to complete a consent discussion via the telephone. Their questions and answers will be recorded. Participants can elect not to participate and may withdraw at any time without penalty. Participants will receive a copy of the combined consent and HIPAA form for their records. In addition, participants will be given the Principal Investigator's and Study Physician's contact information (located on pg.1 of the consent) should they wish to speak to the Investigator or Study Physician during the course of the study regarding their consent or the study procedures. The consent process will take place in English, there will be no waiting period, no coercion to participate, and all participants will be considered competent to provide informed consent (i.e., they will be asked if they understand what they are consenting for). The consent form must be signed and dated by the participant and the investigator-designated research professional obtaining the consent. The original signed combined consent and HIPAA form will be centrally stored in regulatory binders (consent).

12 RESOURCES NECESSARY FOR HUMAN RESEARCH PROTECTION

12.1 Research Staff

The following research staff will be directly involved with the implementation and execution of the current study:

- Janet Audrain-McGovern, Ph.D., Principal Investigator
- Frank Leone, M.D., Study Physician
- E. Paul Wileyto, Ph.D., Collaborating Investigator and Biostatistician
- Rebecca Ashare, Ph.D., Collaborating Investigator
- Kenneth Perkins, Ph.D., Collaborating Investigator (University of Pittsburgh)
- James Loughead, Ph.D., Neuroimaging Sub-Study Collaborating Investigator
- Kyle Bittinger, Ph.D., Microbiome Sub-Study Collaborating Investigator
- Christopher Petucci, Ph.D., Microbiome Sub-Study Collaborating Investigator
- John Detre, M.D., Radiologist
- Susan Ware, Database Developer/Manager
- Paul Sanborn, Research Staff
- Alexa Mazur, Research Staff
- Stephen Pianin, Research Staff
- Wen Cao, Research Staff
- Shannon Testa, Research Staff

- Jazmin Ricks, Research Staff
- Kiera Zehner, Research Staff

12.2 Study Facilities

This project will be conducted at and through the CIRNA. The CIRNA has successfully conducted similar protocols and has well-developed procedures for staff training, data collection and storage, and study completion. The facilities available for this project include a large and small conference room, individual consulting rooms with computer/internet access, storage rooms, office space for study personnel, and data management facilities. In addition, CIRNA houses two freezers for sample storage. A -80°C freezer is used for long-term sample storage, while a -30°C freezer is utilized for daily access of current sample boxes. These freezers contain temperature and power monitoring sensors which are connected to a Sensaphone alarm system that will contact specific biospecimen staff in the event of an emergency.

If participants require referral for psychological services, information about such programs at 3535 Market Street and/or the Philadelphia area will be provided; we have a form with specific information about such programs already in use in other CIRNA studies.

The Center for Functional Neuroimaging (CfN) provides infrastructure support for functional neuroimaging and is comprised of investigators and staff with a broad range of experience including regulatory affairs pertaining to neuroimaging, MRI methods development, MRI physics and pulse programming, instrumentation, experimental design, computing and image analysis procedures. The center provides support for technical aspects of neuroimaging using MRI, including experimental design, data acquisition, and image analysis, and interfaces with several other complementary programs in brain, behavior and imaging. The CfN currently has a computing cluster of over 600 computing cores at 3.1 GHz on a 10GbE network linked to public terminals by a high speed network along with 100TB of RAID storage and tape backup. This cluster runs Matlab, IDL, AFNI, and a variety of customized software environments. The CfN is supported by an NINDS P30 Center Core. The CfN also manages access to neuroscience MRI scanning on research dedicated systems to be used in this trial: A 3 Tesla Siemens Trio whole-body MRI system located in the Hospital of the University of Pennsylvania and a Siemens Prisma 3 Tesla whole-body MRI with a 64-channel head/neck array located in the basement of the Stellar Chance Building.

13 Study Finances

13.1 Funding Source – Parent (Main) Study

This study is financed through a grant from the U.S. National Cancer Institute.

13.2 Funding Source – Neuroimaging Sub-Study

The neuroimaging sub-study is financed through a supplement grant from the U.S. National Cancer Institute.

13.3 Funding Source – Microbiome Sub-Study

The microbiome sub-study is financed through a supplement grant from the U.S. National Cancer Institute.

13.4 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict

reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

13.5 Subject Compensation

Participants will be compensated in cash at each in-person visit they attend. When the neuroimaging sub-study is actively recruiting, participants enrolled in the sub-study may receive up to $730.00 including travel reimbursement for successfully completing all study requirements in their entirety as per the Study Compensation table (Table 3) below. When the microbiome sub-study is actively recruiting, participants enrolled in the sub-study may receive up to $590.00 including travel reimbursement for successfully completing all study requirements in their entirety as per the Study Compensation table (Table 3) below. When neither the neuroimaging sub-study nor microbiome sub-study are recruiting, participants enrolled in the main study may receive up to $490.00 for successfully completing all study requirements in their entirety. No dietary recall compensation will be provided if a participant fails to complete any of the three 24-hour dietary recalls at each of the five assigned time points. Compensation that is earned for completing the 24-hour dietary recall assessments will be distributed in-person or via check (mailed) at the participant's discretion. Participants can earn up to an additional $4.00 during the completion of the RRVF task (up to $1.00 per task attempt). Moreover, participants enrolled in the neuroimaging sub-study may receive up to an additional $4.00 based upon choices made while completing the in-scanner Food Reinforcement Task (up to $2.00 for each Scan Visit).

Participants who are found ineligible for any reason during the Intake Visit or prior to entering the MRI scanner during Scan Visits (neuroimaging sub-study) will only receive travel reimbursement ($5.00). No study compensation will be distributed to participants who complete visit activities over the telephone. Participants will be asked to complete a W-9 tax form at the conclusion of the Intake Visit because the University of Pennsylvania is required to report to the Internal Revenue Service (IRS) any cumulative payments for participation in research studies at the University of Pennsylvania that exceed a total of $600.00 in a calendar year. A W-9 will aid the Center in tracking and reporting those who participate in multiple projects at the Center and accrue over $600.00 in a calendar year.

Table 3. Study Compensation

Study Time Point/ Activity	Compensation	Completion Bonus	Travel Reimbursement	Total
Intake	$20.00	N/A	$5.00	$25.00
fMRI Scan 1[1]	$90.00		$5.00	$95.00
Baseline (Wk -2) Stool Sample [2]	$25.00			$25.00
Baseline	$20.00		$5.00	$25.00
24-Hour Dietary Recalls (3) [4]	$30.00		N/A	$30.00
Pre-Quit	$20.00		$5.00	$25.00
Target Quit Day	$20.00		$5.00	$25.00
Mid-Treatment #1	$20.00		$5.00	$25.00
Mid-Treatment #2	$20.00		$5.00	$25.00
Mid-Treatment #3 (Wk 4) Stool Sample [2]	$25.00			$25.00
Mid-Treatment #3	$20.00		$5.00	$25.00
24-Hour Dietary Recalls (3) [4]	$30.00		N/A	$30.00
Mid-Treatment #4	$20.00		$5.00	$25.00
fMRI Scan 2 [1]	$90.00	$50.00 [5]	$5.00	$145.00
End of Treatment (Wk 8) Stool Sample [2]	$25.00	$25.00 [3]	N/A	$50.00
End of Treatment	$35.00	N/A	$5.00	$40.00
24-Hour Dietary Recalls (3) [4]	$30.00		N/A	$30.00
Follow-Up #1	$45.00		$5.00	$50.00
24-Hour Dietary Recalls (3) [4]	$30.00		N/A	$30.00
Follow-Up #2	$45.00		$5.00	$50.00
24-Hour Dietary Recalls (3) [4]	$30.00		N/A	$30.00
TOTALS			**Main + fMRI Sub-Study**	Up to $730 [6, 7]
			Main + Microbiome Sub-Study	Up to $590 [7]
			Main Study	Up to $490 [7]

[1] Only applicable to fMRI eligible participants who elect to participate in the neuroimaging sub-study (when actively accruing).
[2] Only applicable to participants who are both eligible and elect to participate in the microbiome sub-study (when actively accruing).
[3] Participants who return all three stool samples per study instructions are eligible to receive a $25.00 bonus.
[4] Participants must complete all three 24-hour dietary recall assessments to receive compensation ($30.00) at each assigned time point.
[5] Eligible participants who elect to participate in the neuroimaging sub-study (when actively accruing) and complete both fMRI scans will receive a fifty-dollar completion bonus distributed via check in the mail in addition to the $95 in cash received after completing Scan 2.
[6] Total does NOT include the in-scanner Food Reward Reinforcement/Choice Task (up to an additional $2.00 for each Scan Visit).
[7] Total does NOT include payment that may be earned during the RRVF task (up to an additional $4.00).

14 References

See the NCI parent, neuroimaging supplement, and microbiome supplement grant proposals for references.