Exenatide Once Weekly for Smoking Cessation: A Randomized Clinical Trial

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Abstract

Background: Cigarette smoking is the greatest preventable cause of morbidity and premature mortality in the US. Approved pharmacological treatments for smoking cessation are marginally effective, underscoring the need for improved pharmacotherapies. Glucagon-like peptide-1 (GLP-1) is produced in the intestinal L-cells and in the hindbrain. The peptide maintains glucose homeostasis and reduces food intake. Several GLP-1 agonists are used clinically for the treatment of type 2 diabetes and obesity. In addition to reducing food intake, GLP-1 agonists have recently been shown in preclinical studies to attenuate appetite for drugs and alcohol.

Aims: Our primary aims are: 1) to examine the impact of treatment with weekly extended-release injectable exenatide on smoking abstinence and 2) to assess whether exenatide treatment is associated with the reduction in craving and withdrawal symptoms in the study sample. Our secondary aims are: 1) to examine the effect of extended-release exenatide on cue-induced craving for cigarettes, 2) to evaluate the effect of exenatide treatment on continued abstinence from smoking and craving for cigarettes at 1- and 4- weeks post-treatment, and 3) to collect DNA samples from the study participants for the examination of the GLP-1 receptor variability and smoking outcomes.

Methods: We will enroll pre-diabetic and/or overweight smokers (n=90) who desire to quit smoking. Participants will be randomized 1:1 to receive once weekly exenatide or placebo for a total of 6 weeks. All participants will receive transdermal nicotine replacement therapy (NRT) and behavioral counseling. Abstinence from smoking, craving, and withdrawal symptoms will be assessed during and at 6 weeks of treatment, as well as at 1- and 4-week post-treatment.

Expected Outcomes: We hypothesize that: 1) exenatide treatment will increase the number of participants able to achieve complete smoking abstinence above that achieved via standard NRT and 2) exenatide will reduce craving and withdrawal symptoms, as well as cue-induced craving. Craving and withdrawal symptoms predict long-term efficacy, which could be examined in a follow-up study.

A. Background

Cigarette smoking is the leading cause of *preventable* mortality in the U.S., producing over 400,000 deaths annually[1]. Additional 50,000 deaths occur each year among nonsmokers who are exposed to secondhand smoke. Over 16 million Americans suffer from chronic diseases caused by smoking. Annually, these diseases cost the U.S. $170 billion in direct medical care and over $156 billion in lost productivity [1]. These data illustrate the significance of cigarette smoking on individuals and our society at large and underscore the importance of identifying therapeutic approaches to assist smoking cessation.

The first line pharmacological therapies for smoking cessation include nicotine replacement therapy (NRT), bupropion hydrochloride, and varenicline tartrate. Nearly 50 million of Americans smoke cigarettes, and although many report wanting to quit, the 12-month abstinence rates with the use of these therapies range from 22% to 33% [1]. <u>These relatively modest long-term improvements in abstinence rates underscore the importance of developing improved therapeutic approaches to assist smoking cessation</u> [2, 3].

Glucagon-like peptide-1 (GLP-1) is produced endogenously in the intestinal L-cells and in the hindbrain nucleus tractus solitaries [4-7] in response to nutrient ingestion. Acting primarily via GLP-1 receptors (GLP-1R) in the hypothalamus and brain stem, GLP-1 regulates glucose homeostasis via increasing the secretion of insulin [8] and suppressing the secretion of glucagon [9, 10]. GLP-1 also reduces food intake [11, 12] in part by reducing appetite [13-15]. The proposed study medication, exenatide, a GLP-1 receptor agonist (GLP-1RA), is currently used clinically for the treatment of type 2 diabetes mellitus (DM), and a related GLP-1RA (liraglutide) was recently FDA-approved for the treatment of obesity in patients with or without DM, suggesting that exenatide may have similar effects.

GLP-1Rs are expressed in many brain regions. In addition to hypothalamus and brain stem, GLP-1Rs are expressed throughout the mesolimbic dopamine system [16], and GLP-1 containing neurons extend directly into the ventral tegmental area and nucleus accumbens [17] - areas that are intimately associated with the regulation of reward. These data suggest that the role of GLP-1 extends beyond the glucoregulatory effects to reward regulation and that the GLP-1 system represents a target for pharmacological treatment of addictive behaviors [11, 12]. Consistent with this, recent and novel findings from animal studies have indicated that treatment with GLP-1RAs attenuates reward induced by alcohol [18, 19], cocaine [20, 21], amphetamine [20, 22], and most relevant here, nicotine [23]. In mice, treatment with a GLP-1RA at a dose with no other behavioral effects attenuated nicotine-induced locomotor stimulation, accumbal dopamine release, and reduced the expression of nicotine conditioned place preference [23].

Of additional relevance to the present application, a number of recent studies aimed to evaluate the effects of GLP-1RAs on weight loss using the pharmacogenetic approach [24-26]. The objective of these studies was to examine whether GLP-1R variability moderates the effect of GLP-1RA treatment on weight loss. The studies showed that GLP-1R variability is indeed associated with weight loss in GLP-1RA-treated participants. Several GLP-1R polymorphisms, with the most commonly cited rs6923761, predicted weight response to GLP-1RA treatment [24-26]. Given the overlap among food and drugs of abuse as it relates to engaging the reward circuitry [27, 28], the findings of these studies suggest that GLP-1R variability may moderate the effect of GLP-1RA treatment on smoking outcomes.

B. Research Aims

We will evaluate, for the first time, the impact of treatment with extended-release injectable exenatide on smoking abstinence among pre-diabetic and/or overweight adults presenting for treatment to the UTHealth Center for Neurobehavioral Research on Addiction. In addition, to obtain preliminary evidence for a larger follow-up study that will evaluate the mediating effects of craving and withdrawal symptoms on abstinence in smokers treated with exenatide, we will assess the impact of exenatide on post-quit craving and withdrawal symptoms – proximal factors which covary with and predict future abstinence [29-32]. Lastly, we will collect DNA samples from the study participants to examine the relationship between GLP-1R variability and smoking outcomes.

Primary Aims:

Aim 1: to examine the effect of weekly extended-release injectable exenatide treatment on carbon-monoxide confirmed self-reported smoking abstinence in pre-diabetic and/or overweight smokers following a quit attempt

Hypothesis 1.a.: Exenatide will increase the rate of complete smoking abstinence measured by seven-day point prevalence abstinence (self-reported and biochemically verified via expired carbon-monoxide levels) following six weeks of treatment.

Hypothesis 1.b.: Exenatide-treated participants will show differential change over time in the probability of abstinence as measured by seven-day point prevalence during the six week treatment.

Aim 2: to examine the effect of weekly extended-release injectable exenatide treatment on post-quit craving and withdrawal symptoms in the study sample

Hypothesis 2.a. Exenatide will reduce post-quit craving (as measured by the Questionnaire of Smoking Urges score) and withdrawal symptoms (as measured by the Wisconsin Smoking Withdrawal Scale score) following six weeks of treatment.

Hypothesis 2.b.: Exenatide-treated participants will show differential reduction in post-quit craving and withdrawal during the six week treatment.

Secondary Aims:

Aim 1: To examine the effect of the 6-week exenatide treatment on 1- and 4- week post-treatment abstinence from smoking and craving for cigarettes in the study participants

Hypothesis: Exenatide will increase the rate of complete smoking abstinence measured by seven-day point prevalence abstinence (self-reported and biochemically verified via expired carbon-monoxide levels) at 1- and 4-weeks post-treatment.

Aim 2: To examine the effect of extended-release exenatide on cue-induced craving for cigarettes

Hypothesis: Exenatide treated participants will display lower craving for cigarettes (as measured by the Questionnaire of Smoking Urges score) following virtual reality exposure.

Aim 3: To collect blood samples from the study participants and genotype for GLP-1R polymorphisms and other polymorphisms in the future.

C. Study Design and Methods

C1. General Study Design and Procedures

Our aims will be accomplished via a double-blind, placebo-controlled, randomized clinical trial. The participants will be 90 adult smokers who have pre-diabetes (Type 2, HbA1C=5.7-6.4%) or are overweight (BMI≥25 kg/m^2). The participants will be randomized 1:1 to receive exenatide or placebo. All participants will receive transdermal NRT and individual behavioral smoking cessation counseling. Quit date will be set following 2 weeks of exenatide/placebo treatment because we anticipate that it will take 2 weeks for the effects of exenatide to emerge [33, 34]. Smoking abstinence will be evaluated via self-report and confirmed during clinic visits via breath carbon monoxide (CO) measurements.

Table 1. Study Procedures

Study Timeline	**Screen**	**Weeks 1-2**	**Quit day**	**Weeks 3-6**	**End of Treatment**	**Week 7**	**Week 10**
Smoke?	*Ad Libitum*	*Ad Libitum*		*No Smoking*		*No Smoking*	*No Smoking*
Informed Consent, MINI, Demographics, Inclusion/Exclusion Criteria, Physical Exam, Medical History	X						
Blood (DNA) sample		M, Week 1					
Concomitant Meds, AEs, Vitals	X	M		M		M	M
FTND, Pregnancy Test, Safety Labs	X						
Exenatide or Placebo		M		M			
NRT				M			
Behavioral Therapy		M		M			
Number of Cigarettes Smoked	X	M		M, T, W, T, F, S, S		M	M
Breath CO	X	M		M		M	M
WSWS, QSU, PANAS	X	M		M		M	M
Virtual Reality Session		M, Week 1		M, Week 3			

AEs-Adverse Events; CO-carbon monoxide; FTND-Fagerstrom Test for Nicotine Dependence; M- Monday; MINI-MINI Neuropsychiatric interview, NRT – nicotine replacement therapy; PANAS- Positive and Negative Affect Schedule; QSU – Questionnaire of Smoking Urges, WSWS – Wisconsin Smoking Withdrawal Scale

Design Rationale. Administration of exenatide 2 mg once weekly results in therapeutic concentrations (>50 pg/mL) in approximately 2 weeks, whereas steady-state concentrations of approximately 300 pg/mL are achieved within approximately 4-6 weeks [33, 34]. Based on these pharmacokinetics, we selected to set the quit date following 2 weeks of exenatide treatment and to evaluate the primary outcome (7-day point prevalence abstinence) following a total of 6 weeks of treatment. This is the first study to evaluate the effect of exenatide treatment on smoking outcomes; therefore, the optimal time-points for quit date and assessment of the primary outcome cannot be predicted with certainty. To mitigate this concern, we will evaluate the trajectory of quit attempts and abstinence throughout the duration of the study.

C2. Setting and Treatments

This study will be conducted at the UTHealth Center for Neurobehavioral Research on Addiction. Participants will be recruited using various strategies, including flyers, newspaper ads, and referrals. When the potential participants call the provided telephone number, the requirements and the procedures of the study will be explained, and a brief prescreen to determine initial eligibility will be administered. The pre-screen interview will include questions concerning medical and psychiatric history, psychoactive substance use, and smoking history. Individuals who appear eligible based on the initial pre-screen will be invited for a face-to-face interview.

During a face-to-face interview with the study PI, participants will be given consent documents that will be read to them if needed. Following completion of the informed consent process, each participant will provide his or her medical history and have a brief physical exam including assessment of vital signs and collection of samples for laboratory testing. Following completion of the initial screening and determination of the study eligibility, participants will return to clinic for randomization (1:1) to receive exenatide treatment or placebo. The first dose of exenatide/placebo will be administered during that visit. Subsequent to that visit, participants will return to clinic once a week for 5 more weeks. A total of 6 weekly injections will thus be administered. All participants will receive NRT treatment and individual behavioral smoking cessation counseling as is the recommended standard for use with pharmacotherapy. Cue-induced craving for cigarettes is assessed during visit 1 (baseline) and visit 3 following virtual reality exposure. Participants receive compensation at the end of each study visit.

Upon the completion of the treatment phase of the study, the participants who were abstinent following 6 weeks of treatment will be contacted via telephone at 1- and 4-weeks post-treatment to ascertain continued abstinence from smoking. Those who report being abstinent, will be invited for in-person visit for the biochemical verification of abstinence.

The blood samples for DNA extraction will be collected during the participants' first study visit, using two 5-ml lavender top vacutainers. The samples will be labeled using unique study identifiers and transported at room temperature to the Baylor College of Medicine Psychiatric Genetics Laboratory in the Department of Psychiatry and Behavioral Sciences for further analysis.

C3. Recruitment, Eligibility, and Randomization

Recruitment. We will use advertisements in local newspapers, flyers, as well as referrals to recruit volunteers.

Eligibility. The following inclusion criteria must be met for an individual to participate in the study:

1. Be English-speaking volunteers who desire to quit smoking and are willing to make a quit attempt during the course of the study;
2. Be aged between 18 and 75 years;
3. Have smoked ≥10 cigarettes a day for at least one year and provide a breath CO ≥10 ppm;
4. Have a negative pregnancy test, if female of childbearing potential;
5. Have HbA1C levels between 5.7 and 6.4% and/or body mass index of ≥25 kg/m^2.
6. Not currently using any therapy for glycemic control (either injectable [i.e. insulin] or oral agents);
7. Have vital signs as follows: resting pulse between 50 and 95 bpm, BP between 90-150 mmHg systolic and 45-95 mmHg diastolic;
8. Have hematology and chemistry laboratory tests that are within reference limits (±10%), with the following exception: pancreatic tests (lipase and amylase) must be within normal limits;
9. Have a medical history and brief physical examination demonstrating no clinically significant contraindications for study participation, in the judgment of the principal investigator.

Exclusion Criteria:

1. Meet criteria for the following psychiatric and/or substance use disorders as assessed by the MINI: items C (current manic or hypomanic episode only), I (alcohol abuse - Alcohol Addendum-past 3 months only; current alcohol dependence), J (substance abuse -Substance Abuse Addendum – past 3 months only; current substance dependence), K (current psychotic disorder or current mood disorder with psychotic features).
2. Individuals who meet criteria for non-exclusionary psychiatric disorders that are considered clinically unstable and/or unsuitable to participate as determined by the Principal Investigator.
3. Individuals rated as moderate (9-16) to high (17 or greater) on suicidality as assessed by Module B of the MINI.
4. Have personal or family history of medullary thyroid carcinoma or multiple endocrine neoplasia syndrome type 2;
5. Have type 1 diabetes mellitus;
6. Have severe cardiovascular disease (history of myocardial infarction, life-threatening arrhythmia, or worsening angina pectoris);
7. Have active temporomandibular joint disease;
8. Have severe gastrointestinal disease (i.e. severe gastroparesis);
9. Have previous history of pancreatitis or are at risk for pancreatitis;
10. Have CrCl<30;
11. Have any previous medically adverse reaction to study medications, nicotine, or menthol;
12. Be pregnant or lactating or unwilling to provide a negative pregnancy test before study entry;
13. Not using a reliable form of contraception (e.g., abstinence, birth control pills, intrauterine device, condoms, or spermicide);

14. Have any illness which in the opinion of the primary investigator would preclude safe and/or successful completion of the study.

Criteria for withholding study treatment: Subsequent doses of exenatide will not be administered if 1) any of the following occur: hypoglycemia, hypersensitivity reaction, anaphylaxis, nephrotoxicity, pancreatitis, or severe injection site reaction or 2) the study PI believes that there may be any reason to withhold exenatide. *Criteria for participant discontinuation following study initiation.* Inability to comply with study procedures; meet discontinuation criteria due to side effects/adverse events associated with the study treatment. *Stopping criteria.* We will monitor the frequency of the treatment-related adverse events. If more than 10% of participants entering the study experience treatment-related adverse event, we will halt the study if indicated by the pattern and severity of adverse events. *Drop out.* We plan to recruit a total of 90 subjects and anticipate that 74 subjects will complete the study (80% retention). *Randomization*. An independent investigator will randomize participants 1:1 into exenatide and placebo groups using blocks of four. Sterile saline will serve as the placebo for exenatide. *Exenatide dosing, accounting and observation*. Exenatide will be administered at a dose of 2 mg subcutaneously once a week on Mondays. Any unused medication will be accounted for and disposed of. *NRT dosing, accounting and observation*. NRT will be administered as a transdermal patch at a dose of 21 mg. The patches will be dispensed on Mondays in the amount sufficient for 1 week of use. Any unused patches with be accounted for and disposed of. *Payment to Research Participants.* Participants will receive compensation for their time and effort. Participants will be compensated at the end of the screening visit and at the end of each study visit, regardless of whether they remain in the study. The compensation rate will be as follows:

Screen: $10
Visit 1: $10
Visit 2: $15
Visit 3: $20
Visit 4: $25
Visit 5: $30
Visit 6: $50
Post-treatment Visit 1: $10
Post-treatment Visit 2: $10
Provision of DNA sample: $20

C4. Data Collection and Measures

Clinical Assessments

The MINI Neuropsychiatric Interview will be administered during screening to determine whether subjects meet the DSM-5 criteria for nicotine-use disorder and to rule out presence of any exclusionary psychiatric and substance abuse disorders [35].

Nicotine dependence will be assessed during screening using Fagerstrom Test for Nicotine Dependence [36]. FTND is a valid and reliable 6-items questionnaire that evaluates the quantity of cigarette consumption, the compulsion to use, and dependence.

Baseline smoking status will be assessed during screening via self-reported smoking of >10 cigarettes/day for >1 year and with recent smoking confirmed by an expired air carbon monoxide (CO, Micro$^+$ Smokerlyzer®, Williamsburg, VA) level of ≥10 ppm.

Abstinence from smoking. Self-report abstinence will be logged by participants and confirmed via time-line follow back procedures [37]. Biochemical verification of abstinence (expired CO level of <5 ppm) will be performed during clinic visits.

Withdrawal symptoms will be assessed using Wisconsin Scale of Withdrawal Symptoms (WSWS) [38]. WSWS is a valid and reliable questionnaire which includes subscales of anger, anxiety, concentration, craving, hunger, sadness, and sleep.

Craving for smoking will be evaluated using the Questionnaire of Smoking Urges [39], a 32-item scale that assesses the intention and desire to smoke and an anticipation of relief from the withdrawal-associated negative affect.

Positive and negative affect will be assessed using the Positive and Negative Affect Schedule [40] (PANAS), a 20-item questionnaire that asks participants to rate the extent to which they experience positive (i.e. excited, inspired, proud) or negative (i.e. irritable, distressed, upset) emotions.

Depressive symptoms will be assessed using the Patient Health Questionnaire Depression Scale (PHQ-8) [41], an 8-item questionnaire that assesses presence and severity of depressive symptoms over the previous two weeks.

Virtual Reality (cue-induced craving for cigarettes). To assess baseline withdrawal symptoms and craving for cigarettes participants complete the Wisconsin Scale of Withdrawal Symptoms (WSWS) and the Questionnaire of Smoking Urges (QSU). Participants will then take part in a virtual reality (VR) session that is viewed using a head-mounted device (NIVS SX-60, NIVS, Reston, VA) with a head tracker (Ic3 inertia cube, Intersense) connected to a desktop computer. Participants are exposed to VR neutral cues (nature scenes), followed by series of VR smoking-related cues. VR smoking cues are personalized to match each participant's preferred brand of cigarettes. Similarly, the style of music played in the VR party contexts is also adjusted based on participant preference. Cue reactivity is assessed within the VR environment immediately after each VR scenario with the aid of a gamepad to answer craving-related questions (using a visual analog scale of 0-100). VR neutral context consists of a nature video; VR active paraphernalia context consists of proximal smoking-related objects (packs of cigarettes, ashtrays, bottles of alcohol) placed on tables around the room; and VR party context consists of similar smoking cues and includes interactions with avatars at the party where cigarettes are offered, drinks are poured, and conversations are held while people light up cigarettes. Exposure to each VR environment (neutral, paraphernalia, party) lasts approximately 3 minutes and follows a timed path where at certain points participants are visually directed for a few seconds to pay attention to non-smoking or active cues in order to standardize some of the VR exposure within the environment. This VR paradigm was used in a previous study that showed that self-reported levels of "craving" ($p<0.01$) and "thinking about cigarettes" ($p<0.0001$) were significantly greater after exposure to the active cues versus non-smoking cues and that there were significant positive correlations between self-reported craving prior to the VR session and craving induced by active VR cues ($p<0.01$) [42].

Medical/Pharmacological Assessments

Medical history and physical exam. Personal and family health profiles and medical history will be collected during screening and reviewed with a licensed medical professional. During this time, a physical exam, neurological exam, and neuropsychiatric mental status evaluation will also be conducted.

Clinical laboratory assessment. During screening, blood for hematology studies will be collected in anticoagulant-containing vacutainer tubes for analysis of Hemoglobin A1C, CBC, chemistry, liver function tests, renal function tests, and amylase and lipase levels. A urine-based pregnancy test for females will measure human chorionic gonadotropin. Blood glucose levels will be assessed using finger stick prior to each dose of exenatide.

Blood samples for DNA extraction. The blood samples will be collected during the participants' first study visit, using two 5-ml lavender top vacutainers. The samples will be labeled using unique study identifiers and transported at room temperature to the Baylor College of Medicine Psychiatric Genetics Laboratory in the Department of Psychiatry and Behavioral Sciences for further analysis.

C5. Study Medications

Exenatide. Exenatide will be administered at a dose of 2 mg subcutaneously once a week for 6 weeks. Because administration of exenatide 2 mg once weekly results in therapeutic concentrations (>50 pg/mL) after 2 weeks of treatment, the participants randomized to exenatide treatment will receive 2 weekly doses of exenatide prior to quit day. Because steady-state concentrations are achieved within 4-6 weeks, the participants will be treated for a total of 6 weeks [33, 34]. As noted above, this is the first study to examine the effect of exenatide treatment on smoking outcomes; therefore, the selected time-points for quit date and assessment of the primary outcome may not be optimal. To mitigate this uncertainty, we will evaluate the trajectory of quit attempts and abstinence throughout the duration of the study.

Exenatide will be purchased commercially as Bydureon for SC injection. The incidence of hypoglycemia with the use exenatide is low because the release of insulin following administration exenatide is glucose-mediated. Although there have been questions about the safety of incretins such as exenatide, as treatment may be associated with increased rates of pancreatitis, a recent meta-analysis of 60 studies (n=353,639), consisting of 55 randomized controlled trials (n=33,350) and five observational studies (three retrospective cohort studies, and two case-control studies; n=320,289) has shown that incretin treatment was not associated with increased rates of pancreatitis [43]. Ongoing post-marketing monitoring is also underway. In addition, data from rodent studies suggest that GLP-1 agonists may be associated with an increased risk of thyroid C-cell hyperplasia and C-cell tumors; however, experiments with monkeys did not show proliferation of C-cells in thyroid gland after chronic administration of GLP-1 agonists. Longitudinal data from clinical trials have not demonstrated a causal association between GLP-1 agonists and thyroid C-cell pathology over a 2-year period [44].

NRT. NRT patches (21 mg) will be purchased commercially as generic nicotine patches. The patches will be dispensed on Mondays in the amount sufficient for 1 week of use [45]. Nicotine patches is an over-thecount product with a proven safety record. The patches are contraindicated in persons with hypersensitivity to nicotine or menthol, those with severe cardiovascular disease (i.e. history of myocardial infarction, life-threatening arrhythmia, or worsening angina pectoris), and those with temporomandibular joint disease. To address these concerns, patients with these conditions will be excluded from participation in this study.

C6. Behavioral Counseling

All participants will receive brief individual behavioral smoking cessation counseling as is the recommended standard for use with pharmacotherapy [46]. The counseling protocol is manual driven, has been used in several previous studies [47-49] and will consist of 6 in-person sessions (on the days when the participants come to clinic to receive exenatide/placebo treatment) and 2 brief supportive phone calls (one day pre-quit and 3 days post-quit), lasting 10-15 minutes each, spanning the 6 week active treatment phase. Counseling content will follow the previous study [49] and briefly involves preparation for quitting, identification of high risk situations for smoking, development of coping skills and direct support before and after the quit date, motivational intervention for keeping or resetting a quit date, management of withdrawal symptoms and medication compliance. Counseling will be provided by the study PI, actively practicing Family Nurse Practitioner with a Ph.D. degree. The study PI will be trained by experienced Ph.D. or Master's - level smoking cessation clinicians from Dr. Cinciripini's team. The team has extensive experience from the previous trials in training and monitoring the integrity of the counseling protocol including recording time and content of each session. At the conclusion of active treatment, participants will be offered referral for ongoing cessation counseling (quitline).

C7. Safety Assurances and Adverse Events

Medical Monitoring. Vital signs will be assessed at each visit. Blood glucose levels will be assessed prior to each dose of exenatide. The PI will halt exenatide administration if stopping criteria are met. The dose of exenatide that we will use is the standard dose that is currently used clinically; therefore, we expect no medical issues to arise. Should a participant become unable to cooperate with study procedures he or she will be replaced.

*Adverse event assessment and management. P*articipants' well-being will be assessed at each visit. Participants will be asked if they are experiencing any discomfort indicating potential side effects. Spontaneously reported symptoms or complaints will be recorded and reported as required if events are classified as serious.

D. Data Analysis Plan

Data Management. Initially, data stripped of all personal identifiers will be collected using paper-and-pen or direct computer entry. The database will then be backed up and a copy will be securely e-mailed to the PI and statistical consultant for analysis. A research assistant trained in quality assurance procedures will thoroughly check data from clinical charts against source documents. Twenty percent of the outcome data will be double-entered. In the event of major discrepancies, outcome data will receive 100% source document verification. We will construct a relational data-base in Microsoft Access that will maintain data integrity by limiting entry codes for respective variables and by preventing orphan records.

Sample Size Justification. We will enroll 90 subjects in this study. Our intention is to conduct the largest study possible, given the budget. In the statistical methods outlined below we describe the data analysis strategy that will be used in order to optimize the information provided by the anticipated sample. Furthermore, data obtained in this study will serve as the preliminary evidence for a larger follow-up study.

Statistical Methods. Whether GLP-1RAs confer benefit in treating nicotine dependence has not been tested in clinical trials. Frequentist statistics that are based on dichotomous null hypothesis-testing (i.e. estimating the probability of observing the data, or data more extreme, given that the null hypothesis is true) would be less informative in the context of this early-phase project. We, therefore, select a Bayesian approach to data analysis. Bayesian probability estimates incorporate prior information about plausible parameter values with the observed data, forming the posterior distribution and thereby, allow estimates of the probability that the true value of the parameter falls in some range. The Bayesian approach will thus allow us to bet on the alternative hypothesis and ascertain the probability that the treatment confers some level of benefit, given the observed data [50]. According to the FDA, Bayesian statistics offer improved methodological efficiency for early-phase projects aimed to "repurpose" existing therapies for new indications [51-53]. Decision-making based on an initial trial of a compound for a new indication is assisted by estimates of the probability of an effect of some specified magnitude, even with small sample sizes [54, 55].

Statistical analysis. An intent-to-treat approach will impute those who drop out of the study being counted as continuing to smoke. Demographic and baseline characteristics of the study participants by group that show correlations with both treatment group and outcomes may be potential confounders and result in analyses including and excluding the salient demographic or baseline characteristic to ascertain the degree to which this is the case. Evidence of confounding will result in reporting both adjusted and unadjusted estimates of treatment effect. For retention, group differences in the time to drop-out t over the 6-week study period as a function of treatment will use Cox Proportional Hazards survival analysis. Data analyses will use generalized linear and multilevel models. For Primary Aim 1 and Secondary Aim 1, logistic regression models (with a log-link to permit estimates of relative risks) will be used to assess the effect of treatment on 7-day point prevalence abstinence following 6 weeks of treatment (Primary Aim 1), as well as at 1- and 4-weeks post-treatment (Secondary Aim 1). For Primary Aim 2 and Secondary Aim 2, mixed models and multilevel models will be used to assess the effect of treatment on craving (QSU score), withdrawal symptoms (WSWS score), and cue-induced craving for cigarettes (QSU score following virtual reality exposure). Lastly, multilevel models will the used to examine the trajectory of abstinence throughout the course of the study. Vague, neutral priors provide a basis for initially evaluating the trial results: for linear Cox proportional hazards and logistic regression, priors for coefficients will take the form ~Normal ($\mu = 0$, $\sigma^2 = 1 \times 10^6$) in the linear, log-hazard and log-relative risk scales. Priors for error or dispersion terms will use ~Gamma(a = 0.001, b = 0.001) and ~Uniform(1,1000) for level one and two effects respectively. Working with Dr. Charles Green, we will investigate the robustness of the resulting posterior distributions using a variety of neutral and pessimistic priors representing vague, weakly-informative, and skeptical informative perspectives. Informative priors will use extant information (e.g. meta-analytic findings [56] for smoking outcomes). Evaluation of posterior distributions will permit statements regarding the probability that effects of varying magnitudes exist, given the data. For the genetic analyses (Secondary Aim 3), the primary phenotype to be used as the measure to treatment response will be assessment of smoking over the 6-week evaluation period, as measured by self-report and corroborated by weekly breath CO measurements. This will match the parent exenatide study endpoints. Variants of the GLP-1R will be genotyped and examined for association with treatment response. For continuous traits (breath CO levels) we will perform one-way ANOVA with the continuous trait as the response variable and genotype as the predictor variable. This will test the null hypothesis that the mean level of the trait is the same for all genotypes. We will use linear regression to adjust for any confounding variables before performing one-way ANOVA. For dichotomous traits (smoking abstinence), chi-square analysis will test the null hypothesis that the trait is independent of genotype. For confounding variables, we will use logistic regressed before chi-square analysis. To correct for the multiple testing we will use Bonferroni adjustment.

E. Potential Difficulties and Solutions

Safety of exenatide. Exenatide does not act like insulin to directly lower blood glucose; therefore, the medication is relatively safe and rarely causes hypoglycemia. There have been concerns that GLP-1 may cause pancreatitis; however, a recent meta-analysis suggests this is not so [43]. There are also data from rodent studies suggesting that GLP-1 agonists are associated with an increased risk of thyroid C-cell hyperplasia and C-cell tumors; however, data from clinical trials have not demonstrated a causal association between GLP-1 agonists and thyroid C-cell pathology [44]. To address the concerns regarding the risk pancreatitis and/or thyroid cancer associated with GLP-1 agonists, potential participants with abnormal levels of pancreatic enzymes, those at risk for pancreatitis, and those with personal and/or family history of thyroid C-cell tumor will be excluded from participation in this study. It is also possible that exenatide may not be tolerated well by the study population. This is unlikely, given that the exenatide is generally well tolerated. If a participant does not tolerate exenatide, subsequent dose of exenatide will not be administered and the participant will be replaced.

Recruitment. Critical to any clinical study is recruitment of sufficient numbers of participants. We anticipate no difficulty recruiting the required sample size. As noted in the Background section, cigarette smoking is a common problem. *Retention.* Should dropout be a problem we will increase subject payment which will increase retention.

F. Interpretation and Potential Importance of Findings

The objective of this early-phase project is to estimate the probability that the proposed treatment (exenatide) confers a clinically relevant level of benefit in treating nicotine dependence. If our hypotheses are confirmed, the results of this early-phase project would provide strong preliminary data in support of a definitive follow-up study. In a follow-up study we would assign subjects to placebo and exenatide with quit dates at 2 and 6 weeks of treatment to determine the onset of clinical effects. We would then increase the study duration to at least 12 weeks. This would enable us to clarify the length of time needed to achieve the therapeutic levels and would provide a clearer test of efficacy. We will also examine the mediating effects of craving and withdrawal symptoms on abstinence, which would elucidate the mechanisms by which GLP-1 agonists enhance smoking abstinence.

Overall, there are at least three aspects of this study that are highly important:

1) This is the first clinical study to evaluate exenatide as potential treatment for nicotine dependence. If this treatment is effective, it could represent a new approach to assist smoking cessation. In addition, because exenatide is unrelated to other smoking cessation therapies, it could be combined with other treatments, such as NRT or varenicline, to create an effective "quit package".
2) If exenatide is effective as treatment for nicotine dependence, smokers with or at risk for DM would require only one treatment modality for glucose control and smoking cessation.
3) Given that fear of weight gain is a frequent reason for smokers' reluctance to quit [57] treatment with exenatide may represent an integrated approach to smoking cessation and post-cessation weight gain in overweight smokers.

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