Official Title in ClinicalTrials.gov: Treatment for Nicotine Addiction in Women

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PROJECT DESCRIPTION

In addition to the Application and the Abstract, briefly describe the research project using the numbered headings below. Give sufficient detail for each of the following points to facilitate local review. Use "none" or "NA" if appropriate. *NOTE: This description is for local review committees only and does not need to have the same technical detail as the narrative portion of an application for funding submitted for outside review. However, the description of involvement of human subjects must not differ in substance from the relevant application.*

1. Principal Investigator:

Elise E. DeVito, Ph.D.

Co-Investigators While all research staff are trained to provide informed consent, those identified with an asterisk (*) will provide informed consent for this study. All the research staff will have access to the PHI

Co-Principal Investigator: Mehmet Sofuoglu, M.D., Ph.D.

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Research Staff: *Stacy Minnix, B.S.W., and *Chris Cryan

2. Purpose:

The proposed trial will use a double-blind, placebo-controlled design to conduct the first randomized controlled trial of the COMT inhibitor, tolcapone, in nicotine dependent women.
Aim #1. To determine if tolcapone is superior to placebo in attenuating the severity of nicotine withdrawal and smoking urges during short-term abstinence. Withdrawal severity will be assessed by a self-report scale and cognitive assessment, including a sustained-attention task. Smoking urges will be assessed by a self-report scale.
Hypothesis #1: Tolcapone will be superior to placebo in reducing the severity of withdrawal and smoking urges during short-term abstinence.
Aim #2. To determine if tolcapone is superior to placebo in reducing smoking self-administration in a human laboratory model (Smoking Choice Paradigm) and reducing subjective ratings of smoking reinforcement.
Hypothesis #2A: In the smoking choice paradigm following short-term abstinence, the group receiving tolcapone will choose fewer cigarette puffs than the group receiving placebo.
Hypothesis #2B: In the smoking choice paradigm following short-term abstinence, the group receiving tolcapone will rate smoking as less reinforcing than the group receiving placebo.

3. Background:

Existing smoking cessation pharmacotherapies may be less effective in women than men (Cepeda-Benito *et al*, 2004), which may contribute to poorer quit rates in women (Perkins, 2009). Women may also have higher risks of tobacco-related morbidity and mortality than men smokers(USDHHS, 2001, 2004). Thus, there is a great need for treatments tailored for women smokers.
There are several reasons why the inhibition of the catechol-O-methyltransferase (COMT) enzyme may be a promising smoking cessation pharmacotherapy in women.

i) High COMT enzyme activity is implicated in risk for nicotine dependence and poor smoking cessation outcomes. Individuals with the high COMT enzyme activity allele (Val), are more likely to smoke(Enoch *et al*, 2006; Shiels *et al*, 2008), have higher risk for nicotine dependence (Tammimaki and Mannisto, 2010), experience more withdrawal symptoms (Loughead *et al*, 2009) and smoking urges (Wang *et al*, 2008)) during nicotine-abstinence, have poorer smoking cessation outcomes(Colilla *et al*, 2005), and have diminished therapeutic effects of nicotine replacement therapy for smoking cessation(Johnstone *et al*, 2007), relative to relative to those with the allele that inhibits COMT enzyme activity (Met).
ii) There are several biologically plausible mechanisms by which COMT enzyme inhibition could counter nicotine dependence. COMT enzyme is abundant in the brain and affects prefrontal cortical (PFC) and striatal dopaminergic systems implicated in nicotine dependence.
The COMT enzyme metabolizes dopamine (DA) (Tammimaki *et al*, 2010). A functional variant of the gene which codes for the COMT enzyme, reduces enzymatic activity (i.e., Met relative Val allele carriers) (Chen *et al*, 2004) results in higher basal DA levels, primarily in the prefrontal cortex, but also in the striatum(Gogos *et al*, 1998; Tammimaki *et al*, 2010; Yavich *et al*, 2007) and lower nicotine-induced DA release in ventral striatal regions(Brody *et al*, 2006). Nicotine-induced striatal DA release increases nicotine's reinforcing and hedonic properties (Barrett *et al*, 2004) and alleviates abstinence-related smoking urges (Brody *et al*, 2004) and mood disturbances (Brody *et al*, 2009). So, the fact that COMT enzyme inhibition reduces nicotine-induced striatal DA release suggests it may also reduce nicotine's reinforcing properties, which should reduce smoking maintenance. Smoking abstinence normally results in reduced basal DA levels in the ventral striatum, which is associated with withdrawal symptoms (Zhang *et al*, 2012). Since inhibition of the COMT enzyme increases basal striatal DA levels, it should diminish abstinence-related withdrawal symptoms. This is important because withdrawal symptoms (e.g., irritability, negative affect) and smoking urges increase relapse-risk during smoking cessation attempts (Allen *et al*, 2009a; Allen *et al*, 2009b; Allen *et al*, 2008b). PFC DA levels influence cognitive function. Abstinence-related cognitive decrements are a core feature of withdrawal(Hughes, 2007) and increase smoking relapse risk (Patterson *et al*, 2010) (de Wit, 2009). As such, cognition has been proposed as a treatment target for smoking cessation(Sofuoglu *et al*, 2013). High COMT enzyme activity is associated with poor performance on cognitive functions implicated in relapse risk (e.g. working memory, sustained attention (Barnett *et al*, 2007; Egan *et al*, 2001) (Diaz-Asper *et al*, 2008; Dumontheil *et al*, 2011; Loughead *et al*, 2009)) and with greater nicotine-abstinence related cognitive decrements(Herman and Sofuoglu, 2010; Loughead *et al*, 2009). Therefore, since COMT enzyme inhibition increases PFC DA levels, it is expected to diminish abstinence-related cognitive decrements, which could reduce relapse-risk since abstinence-related cognitive decrements increase smoking relapse.
iii) Many of the effects of COMT enzyme inhibition may be sex-sensitive. Inhibition of COMT enzyme activity has sex-sensitive effects on psychiatric risk factors(Tunbridge and Harrison, 2010). Such sex-sensitive effects may arise from neurobiological sex differences in DA systems which are regulated by the COMT enzyme (drug-stimulated striatal DA release(Munro *et al*, 2006) and basal striatal DA levels(Laakso *et al*, 2002)); gonadal hormones' modulation of DA (e.g. estradiol's DA agonist-like effects(Becker, 1990a, b, 2000; Becker and Hu, 2008; Pasqualini *et al*, 1995; Thompson and Moss, 1994; Xiao and Becker, 1994)); or gonadal hormone's modulation by COMT (COMT enzyme's catabolism of estrogens(Tunbridge *et al*, 2010)).

For example, COMT enzyme activity's affects on nicotine-abstinence related cognitive decrements are sex-sensitive and moderated by menstrual cycle phase(Jacobs and D'Esposito, 2011). Nicotine withdrawal symptoms and smoking urges may be stronger in women (al'Absi *et al*, 2002; Leventhal *et al*, 2007), symptoms of anxiety and depression are more strongly linked with nicotine withdrawal and smoking relapse in women (Weinberger *et al*, 2009) and high COMT enzyme activity has been more strongly linked with depression, anxiety and related phenotypes in women than men (Baune *et al*, 2008; Domschke *et al*, 2012; Domschke and Dannlowski, 2010; Domschke *et al*, 2007; Domschke *et al*, 2004; Eley *et al*, 2003; Enoch *et al*, 2003; Funke *et al*, 2005; Hamilton *et al*, 2002; Hettema *et al*, 2008; Kempton *et al*, 2009; Kim *et al*, 2006a; Lonsdorf *et al*, 2010; Massat *et al*, 2005; Olsson *et al*, 2005; Opmeer *et al*, 2010; Rothe *et al*, 2006; Stein *et al*, 2005; Weiss *et al*, 2007; Williams *et al*, 2010).
iv) Our preliminary data support our hypotheses in women smokers. We recently demonstrated that overnight-abstinent women smokers with high COMT enzyme activity reported greater severity of certain withdrawal symptoms (e.g., difficulty concentrating) than Met carrier women or men with high or low COMT enzyme activity (Herman *et al*, 2013). Our preliminary data extend these findings in a larger sample to show that in women, but not in men, high COMT enzyme activity is associated with more severe withdrawal, smoking urges and worse cognitive function following overnight abstinence. Additionally, high COMT enzyme activity women rated their anxiety higher during placebo yet reported greater alleviation of anxiety symptoms with nicotine administration. As such, inhibition of COMT enzyme activity would be expected to ameliorate withdrawal symptoms (including negative mood) and smoking urges, particularly in women, providing another mechanism by which this intervention would be expected to improve smoking cessation outcomes in women.
v) Approved medications that inhibit COMT enzyme activity are available for clinical use. COMT enzyme activity can be pharmacologically inhibited with tolcapone, which crosses the blood-brain barrier and inhibits COMT enzyme activity in the brain (Ceravolo *et al*, 2002) to a similar degree as the genetic variant described above (i.e., Met allele; 25% reduction (Apud *et al*, 2007)). While it is approved as an adjunct to DA treatments in Parkinson's disease(Haasio, 2010), tolcapone has been shown to improve aspects of cognitive function in healthy individuals(Apud *et al*, 2007) and patients with Parkinson's disease(Gasparini *et al*, 1997) or Pathological Gambling(Grant *et al*, 2013) and in pre-clinical studies(Liljequist *et al*, 1997; Tunbridge *et al*, 2004). Tolcapone reduced gambling urges and behaviors(Grant *et al*, 2013) and another COMT inhibitor (entacapone) diminished cannabis-craving in dependent individuals(Shafa *et al*, 2009). Taken together, these findings support tolcapone's safety and efficacy in improving symptoms implicated in addiction risk, including cognitive performance and craving in other addicted populations.

4. Significance:
Nicotine dependence has been a persistent public health problem and identifying effective medications for the treatment remains a high priority. Women smokers, compared to men, have lower smoking quit rates (Perkins, 2009), may find smoking cessation medications to be less effective(Cepeda-Benito *et al*, 2004), and may have higher risks of tobacco-related morbidity and mortality (USDHHS, 2001, 2004). Thus, there is a great need for treatments tailored for women smokers. The results will provide important evidence that COMT inhibition is a viable therapeutic target for smoking cessation in women, and contribute to a better understanding of its potential mechanisms of action.

5. Research Plan:

A. Overview

The proposed study will assess the effects of the COMT inhibitor, tolcapone, versus placebo on reducing withdrawal symptoms (including cognitive decrements), smoking urges and smoking behavior, in women smokers. This will be a double-blind, placebo-controlled study, with 60 women smokers. After completing an *ad libitum* smoking baseline, participants will be randomized to 8-days of the COMT inhibitor tolcapone (100mg three times a day (TID) for 7 days then 100mg once on day 8) or placebo (Apud *et al*, 2007; Grant *et al*, 2013). For the first two days of the medication period, participants will continue to smoke *ad libitum* and attend brief check-in sessions once daily to take one dose and be given the remaining two doses to take at home. On medication days 3-5, they will be given take-home doses and will be asked to check in daily by phone or in person and continue to smoke *ad libitum*. On the evening of medication day 5, the 60-hr (2 ½ day) abstinence period will begin. During the 2 ½ day abstinence period, participants will attend brief check-ins daily, to receive their dose (plus take-home doses) and to be screened for abstinence adherence (breath CO levels; urine cotinine). Measures of withdrawal and smoking urges will be collected at all sessions. On Medication Day 8, a test session will be conducted where assessment of cognitive function and smoking behavior will be collected. At the conclusion of the session on Medication Day 8, subjects will be stop the medication. A brief follow-up session will be completed approximately 1-week after the final medication day then subjects will be discharged from the study. A between-subject design will be used since the study involves numerous visits and several days of abstinence and we predict that a within-subject design would be overly burdensome to participants and result in high attrition rates. A between-subject design also avoids possible medication carryover effects.

Subjects: Women smokers, aged 18-45, who are able to read and write English and are in good health (as verified by medical history, screening examination, and screening laboratory tests) will be included. Smoking status will be determined by self-reported history of smoking daily for the past 12 months, ≥5 cigarettes daily, , a urine cotinine of at least 3 (out of 6) as measured by the NicAlert dipstick method to measure smoking status. Subjects will be excluded if they are using other psychotropic medications, have countraindications to tolcapone use based on medical history (including allergy to tolcapone, lifetime history of diagnoses associated with liver disease (e.g., alcohol dependence, hepatitis), renal impairment, history of nontraumatic rhabdomyolysis or hyperpyrexia and confusion possibly related to a medication or lifetime history of syncope), screening examination (e.g., symptomatic orthostatic changes during screening), or screening laboratory tests (e.g., LFT>2 times greater than the upper limit of the normal range)) have other substance use disorders, are pregnant or breast-feeding or not using acceptable birth control methods, have current psychotic disorder, bipolar disorder, homicidal or suicidal ideation, have current (past month) substance use disorder, other than nicotine dependence, or are unable to fulfill the scheduled visits and procedures.

B. Outcome Measures

Table 1. Schedule of Assessments and Interventions	Time (min)	Screen	*Ad Libitum* Baseline	Brief Medication Visits *1visit/day, 4 days*	2 ½ day Abstinence End of Trial	1-week Follow-up
Screen & Baseline Measures & Smoking Behavior Measures						
Psychiatric and Medical Screen	35	X				
Demographics, Menstrual Cycle (MCAQ)	7		X			
Smoking History, Nicotine Dependence(FTND), urine cotinine dipstick	5	X				
Timeline Followback	5		X			X
Pill Administration, Abstinence Adherence and Physiological Measures						
Tolcapone or Placebo	2			X	X	
CO levels, Breathalyzer, urine cotinine	2	X	X	X	X	X
Urine toxicology	3	X	X	X (day 1)	X	
Urine pregnancy screen	3	X		X (day 1)	X	X
Vitals (HR, BP)	2	X	X	X	X	X
Blood Draws						
Genetics (rs4680)	*3*	X				
Labs for liver function	*3*	X			X	X
Nicotine, Cotinine, Hormones	*3*		X		X	
Self-report Measures of Withdrawal, Craving, Drug Effects, Mood and Impulsivity						
Withdrawal (MNWS); Smoking Urges(BQSU)	6		X	X	X	X
Subjective Nicotine Effect (DEQ)	2		X		X	
Adverse Drug Effects (AEF)	3			X	X	X
Depression (CES-D); Anxiety (STAI)	8		X		X	X
Affect (PANAS)	3		X	X	X	X
Impulsivity (BIS-II, BIS/BAS)	7		X			
Cognitive Tasks						
IQ	10	X				
Memory; Learning (digit span, HVLT)	25		X		X	
Sustained Attention (RVP, CPT)	10		X		X	
Cognitive Flexibility (CGT, SOC, Stroop))	10		X		X	
Laboratory Smoking Behavior						
Smoking Choice Paradigm	90				X	
TOTAL ESTIMATED TIME (hours):		***1***	***1 ¾***	***1 ½ (20min/visit)***	***3***	***½***

Subjective/Questionnaires

Mini International Neuropsychiatric Interview (M.I.N.I.): At screening, Axis I psychiatric disorders will be assessed with the Mini International Neuropsychiatric Interview (M.I.N.I.)(Sheehan *et al*, 1998) plus questions for classification by new DSM-V as well as DSM-IV criteria. This assessment will be administered once only at initial screening and will be used to help determine eligibility of the subject to participate in the trial.

Fagerstrom Test of Nicotine Dependence (FTND): This self-report measure FTND (Heatherton *et al*, 1991) will assess degree of nicotine dependence. It will be administered at screening.

Barrett Impulsivity Scale (BIS-11): This self-report measure assesses facets of impulsivity and is designed to measure impulsivity as a 'trait'(Patton *et al*, 1995). The questionnaire is composed of 30 items scored from 1 (rarely/never) to 4 (almost always/always). Impulsivity has been proposed to relate to the development and maintenance of addictive behaviors (de Wit, 2009; Krishnan-Sarin *et al*, 2007; Moeller *et al*, 2001). It will be administered at *ad libitum* baseline.
Behavioral Inhibition/Activation Scale (BIS/BAS): This self-report measure consists of 20 items, scored from 1 (strongly disagree) to 4 (strongly agree) and assesses 'behavioral inhibition' (i.e., anticipation of punishment) and 'behavioral activation' subscales (i.e., reward responsiveness, drive, fun-seeking)(Carver and White, 1994). These traits have been linked with impulsive behaviors and with the development and maintenance of addictive behaviors (de Wit, 2009; Krishnan-Sarin *et al*, 2007; Moeller *et al*, 2001). It will be administered at *ad libitum* baseline.

Menstrual Cycle Assessment (MCAQ): This questionnaire will be administered at *ad libitum* baseline and asks about use of contraception, including hormonal contraception, date of last period, frequency and regularity of cycles and symptoms of menopause or peri-menopause.

Demographics, smoking history: At screening, subjects will also be asked about self-described race and ethnicity as well as years of smoking, age of onset, and past cessation attempts and use of other nicotine products (e.g., e-cigarettes).

Minnesota Nicotine Withdrawal Symptom Checklist (MNWS): At all sessions except screening, smokers will be asked to rate nicotine withdrawal symptoms (cigarette craving, irritability/anger, anxiety/tension, difficulty concentrating, restlessness, increased appetite, depressed mood, and insomnia) on a 100 mm scale, "not at all" to "extremely" (Hughes and Hatsukami, 1986).

Brief Questionnaire on Smoking Urges (BQSU): This 10-item scale reliably measures cigarette craving (Tiffany and Drobes, 1991) and will be administered at all sessions except screening.

Positive and Negative Affect Schedule (PANAS): The PANAS is a 20-item scale that assesses both negative and positive affective states (Watson *et al*, 1988). This scale is sensitive to the affective symptoms of tobacco withdrawal and predicts relapse to smoking (Kenford *et al*, 2002) and will be administered at all sessions except screening.

Premenstrual Assessment Form (PAF): Given their potential overlap with withdrawal symptoms(Allen *et al*, 1996; Allen *et al*, 1999; Carpenter *et al*, 2006), premenstrual symptoms will be assessed with the 10-item PAF (Halbreich *et al*, 1982) at all sessions except screening so they can be used as covariate in analyses focusing on withdrawal symptoms.

Drug Effects Questionnaire (DEQ): Smokers will rate 10 items that are related to nicotine effect on a 100 mm scale, "not at all" to "extremely." The items are feeling "stimulated", "high", "anxious", "sedated", "down", "good drug effects", "bad effects", "drug strength" as well as

"wanting more of the drug" and "liking the drug". This instrument allows rapid detection of nicotine effects, is adapted from a VAS (Soria et al. 1996), and has been used in our prior human laboratory studies with smokers (e.g., (DeVito *et al*, 2013)). This questionnaire will be administered at the *ad libitum* baseline based on the last cigarette they smoked that day (or one they smoke during allowable breaks) and post--abstinence final session based on the cigarette puffs smoked during the Smoking Choice Paradigm.

Center for Epidemiologic Studies Depression (CES-D) scale: The CES-D is a 20-item self-report measure of depressive symptoms (Radloff, 1977)) and will be administered at *ad libitum* baseline and final sessions.

State-Trait Anxiety Inventory (STAI): The STAI is a self-report questionnaire that includes two components: a 20-item component that measures 'trait' anxiety, which is expected to be stable over time, and a 20-item component that measures 'state' anxiety, which is expected to fluctuate across time (Kendall *et al*, 1976). Questions are rated on a 4 point scale from "almost never" to "almost always". The 'state' component will be assessed at *ad libitum* baseline only while the 'trait' component will be included at *ad libitum* baseline and the post--abstinence final session.

Adverse Events Form: The SAFTEE form tracks adverse -effects of the medication and will be tracked at each visit and phone check-in once the medication (or placebo) has begun (Sofuoglu *et al*, 2001; Sofuoglu *et al*, 2005).

Timeline Follow-Back: Retrospective day-by-day self-report of past week smoking and substance use volumes(Robinson *et al*, 2014) will be carried out at the *ad libitum* baseline session and the one week follow-up.

Cognitive

The Shipley Institute of Living Scale (SILS): This is a brief, well validated means of estimating intelligence quotient (IQ) (Zachary, 1991). The form consists of 60 items comprising 2 sub-scales, a vocabulary subscale (40 items) which asks subjects to indicate which of four words is most similar in meaning to the target word, and an abstract thinking subscale (20 items) wherein subjects are asked to fill in a letter or number to complete a pattern. The SILS will be administered at the screening session to check for baseline group differences, since cognitive measures are included as outcomes and are expected to differentially change with withdrawal across medication groups.

Computerized Testing: Cognitive testing, administered at *ad libitum* smoking baseline and the post-2 ½ day-abstinence final assessment, will include computerized tasks of sustained attention (ANAM Continuous Performance Task (CPT)); CANTAB Rapid Visual Information Processing (RVP)), executive function in including cognitive flexibility, cognitive control planning and decision-making (CANTAB Stockings of Cambridge (SOC), CANTAB Cambridge Gamble Task (CGT), ANAM Stroop); and memory/learning (Hopkins Verbal Learning Task (HVLT); Digit Span) (Owen *et al*, 1991) (Owen *et al*, 1990) (Wechsler, 2008) (Brandt, 1991) (Reeves *et al*, 2002). Parallel task versions will be used where available. These tasks were chosen for the cognitive domains' sensitivity to DA(Ersche *et al*, 2011; Harmer *et al*, 2001; Lange *et al*, 1992; Levin *et al*, 2011; Mehta *et al*, 1999; Owen *et al*, 1992), or nicotine(Sahakian *et al*, 1989); engagement of PFC-striatal neurocircuitry (Chase *et al*, 2008; Clark *et al*, 2007; Cools *et al*, 2004; Ersche *et al*, 2011; Kim *et al*, 2006b; London *et al*, 2005; Mehta *et al*, 2000; Owen *et al*,

1991); impairment in smokers(Durazzo *et al*, 2012; Hughes, 2007); relevance to smoking outcomes(de Wit, 2009; Patterson *et al*, 2010); and proposed relevance as treatment targets for smokers(Sofuoglu *et al*, 2013).

Biochemical

Genetics: Blood samples for DNA extraction will be collected at screening to examine whether the Val158Met single nucleotide polymorphism (SNP) of the COMT gene modifies the effects of nicotine. To protect confidentiality, each subject's blood sample will be encoded with a numeric designation and the name of the individual will be stored in a separate database. The samples will be transferred to the Genetic Laboratory at the VA medical Center for processing and storage.
Laboratory Bloods: Standard laboratory blood screening will include liver and thyroid function tests, CBC, serum electrolytes, BUN, creatinine, PT and PTT. This will be done at screening, again at the post-2 ½ day abstinence testing session and at the 1-week follow-up.

Urine screens: Urine pregnancy screens will be administered at screening, *ad libitum* baseline, prior to medication initiation, and prior to Smoking Choice Paradigm at the post-abstinence final session and at the 1-week follow-up. Urine drug screens will be administered at screening, *ad libitum* baseline, prior to medication initiation, and at the post-abstinence final session. Urine cotinine levels will be measured at baseline and check-in sessions and at post-abstinence testing session.

Serum estradiol and progesterone analysis: Serum estradiol and progesterone levels will be measured before the *ad libitum* smoking baseline and post-abstinence final session for use as covariates in our analysis, since female sex hormones may contribute to variation in women's response to nicotine or withdrawal (Allen *et al*, 2009b; DeVito *et al*, 2013) or women's smoking cessation ability (Allen *et al*, 2008a).

Plasma cotinine, 3-hyroxycotinine (3-HC), and nicotine levels: Plasma samples will be taken at the *ad libitum* smoking baseline session and at the post-abstinence final session. 3-HC is the main metabolite of cotinine and the ratio of 3-HC/cotinine, also known as the nicotine metabolite ratio, reflecting the rate of nicotine clearance. Smokers with high 3-HC/cotinine ratio (faster metabolizers) reported greater craving for cigarettes following overnight abstinence and greater nicotine-induced subjective drug effects, suggesting the 3-HC/cotinine ratio may influence the reinforcing effects from nicotine or experience of abstinence. Plasma nicotine levels will also be measured at baseline to confirm smoking status and at the post- abstinence session to confirm abstinence. Assays for nicotine, cotinine, and 3-HC levels will be performed in Dr. Peter Jatlow's laboratory. Briefly, nicotine, cotinine and 3-HC are measured using HPLC coupled to tandem mass spectrometry (LC/MS/MS) employing stable isotope labeled internal standards as previously described.

Alveolar carbon monoxide: A measure of expired CO levels will be taken at the beginning of each visit and during the Smoking Choice Paradigm. At the medication stabilization *ad libitum* check-in sessions, CO readings will be collected as a secondary measure of smoking levels. During the check-in sessions and final session during the abstinence period, CO readings ≤ 8ppm will be used to verify compliance with smoking abstinence on the first morning check-in following overnight abstinence. This level was recommended by the SRNT Subcommittee on

Biochemical Verification to confirm overnight abstinence (Benowitz et al. 2002). At subsequent abstinence check-ins (across the 60 hr abstinence) the subject will be considered to have met the criteria for abstinence-related bonus payment if their CO is ≤ 8ppm and has not increased since their prior abstinence visit.

Physiological
Vitals: Heart rate and blood pressure will be collected once at each visit and again at the end of the Smoking Choice Paradigm.

Behavioral

Smoking Choice Laboratory Paradigm: Nicotine self-administration behavior will be measured with the number of "puffs" from a cigarette chosen during the Smoking Choice Paradigm. This paradigm will be administered once at the post-abstinence visit.

Apparatus: Measures of smoking behavior will be obtained using a smoking topography device. Subjects will smoke through a plastic cigarette holder that is fitted to the filter end of a cigarette and connected to a smoking topography device (CreSS from Plowshare Technologies). The cigarette brand will be the choice of each subject and will be provided by the investigator.

Sample Smoking: The sample smoking will be administered using a Directed Smoking Procedure. In this procedure, the puff duration (3 sec), the interval between 2 puffs (20 sec), and the puff volume (40 cc) are predetermined to minimize the variation in the amount of cigarette smoke inhaled across smoking choices (Zacny *et al*, 1987). The selected puff volumes reflect the average puff volumes in female smokers (Battig *et al*, 1982; Eissenberg *et al*, 1999; Hofer *et al*, 1992). To take puffs of a cigarette, subjects will light a cigarette, without inhaling, and insert it into the cigarette holder. A tone will guide subjects as to when to start and end inhaling each puff. When puff volume reaches the target volume, a tone will sound indicating that the subject should stop inhaling.

Paradigm Procedure: Participants will complete this paradigm in a designated, negative pressure room in our laboratory. The Smoking Choice Laboratory Paradigm will offer participants 2 sample 'puffs' followed by 10 additional smoking opportunities (2 'puffs' per smoking opportunity) spanned across 90 minutes, for a total of 12 "puff" opportunities, wherein tokens can be exchanged for money ($0.75/token) or 2 cigarette puffs. If participants choose cigarette puffs instead of money at every opportunity, they will smoke the equivalent of slightly more than one cigarette within the 90-minute laboratory paradigm.

This paradigm represents a slight adaptation from the Smoking Choice Laboratory Paradigm that our group has previously used by our group (e.g., (Sofuoglu *et al*, 2009)). In prior studies, the 10 puff (in exchange for token) opportunities were spaced by 15 minutes each, therefore spaced over a duration of 2hr15 minutes. In this version the puff opportunities will be spaced by 9 minutes to condense the 10 puff opportunities into 90 minutes, to reduce subject burden. Since the total number of puffs will remain the same as prior protocols and will amount to only slightly more than one cigarette, this dosing remains moderate. If participants self-administer every puff offered, the cigarette will still be spread over much more time than most smokers would use to smoke a single cigarette (which normally lasts 5-10 minutes, rather than 90 minutes).

D. Drugs

Tolcapone: Generic tolcapone will be purchased by the Research Pharmacy from McKesson

Corp. Following oral administration, tolcapone reaches peak plasma levels within 1.5-2.0 hours and maximum inhibition of COMT activity in red blood cells is observed in 1-4 hours (Jorga, 1998; Jorga *et al*, 1998; Mannisto and Kaakkola, 1999).
Tolcapone's primary clinical use is in Parkinson's disease, as an adjunct to dopaminergic medications (e.g., levodopa) to optimize the balance and transitions between "on" and "off" states. In our study, smokers will take tolcapone at 100 mg dose three times a day (TID) for 7 days, and will receive a 100 mg dose on Day 8. Dose administration will be spaced by approximately 6 hours. Medication administration and oversight during the approximately one-week trial will be carefully monitored by our study nurse and physician.

Justification for the tolcapone doses: This dosing schedule is consistent with the manufacturer's recommended use of tolcapone. Previous recent studies with significant effects of the manipulation without problematic adverse drug effects began at 100mg TID for the first day then increased to 200mg TID (Apud *et al*, 2007; Grant *et al*, 2013), so our dosing is more conservative relative to these recent studies. Our dosing schedule is chosen to provide enough time to reach steady-state tolcapone levels (approximately 5 half-lives of tolcapone) prior to initiation of the abstinence period, then provide continued administration during the abstinence period.
Placebo: To maintain the double-blind, placebo pills will be designed to be visually identical to the tolcapone pills, and contain 25mg riboflavin to match the urine discoloration produced by tolcapone.

E. Study Procedures

Subjects will be asked to refrain from consuming alcoholic beverages and drugs during study participation, which will be verified by a urine drug quick test and breathalyzer. If results indicate non-compliance with these study procedures, subjects will be discharged from the study. Subjects will be instructed to drink their typical amount of caffeinated beverages in the morning to minimize caffeine withdrawal, which could confound the study measures. For outline of protocol per session and estimated timings, see Table 1. Briefly, sessions will include:

In person Screening Session: This session will involve: a) obtaining informed consent; b) smoking history and assessment of nicotine dependence severity based on FTND; c) CO levels will be measured ; d) urine cotinine of 3 (out of 6) or higher on the semi-quantitative cotinine dipstick will be measured to ensure smoking status and a urine sample will be collected for quantitative cotinine assay; e) screening for co-morbid psychiatry conditions using the MINI; f) physical examination including ECG and vitals; g) urine analysis for drug screening and urine pregnancy test; h)) blood draws for: 1.) laboratory examination including CBC, ALT, AST, alkaline phosphatase, glucose, BUN, creatinine and 2.) DNA; i) SILS to measure estimated IQ.

Ad libitum Smoking Session: Those found eligible will complete the *ad libitum* smoking baseline assessment, consisting of self-report measures of mood, withdrawal and smoking urges, cognitive tasks and vitals. Biochemical measures will include CO levels, breathalyzer, urine toxicology for drug screening, urine cotinine, blood draws for: 1.) nicotine and nicotine metabolite levels and 2.) gonadal hormone levels (progesterone, estradiol).

Medication Initiation (Medication Days 1-2) and Abstinence Phase (Medication Days 6-7) Sessions: Those subjects who were found eligible will be randomized to medication

(tolcapone) or placebo, in a double-blind fashion. Urn randomization will endeavor to balance medication and placebo groups for race, baseline smoking rate, and menstrual cycle phase. Although genotype will not be known for all subjects prior to randomization, in cases when it is known (e.g., genetics sample collected at baseline is processed prior to randomization) will be counterbalanced for genotype as much as possible.

Brief once-daily visits during the medication initiation (Medication Days 1-2) and abstinence periods (Medication Days 6-7) will serve four purposes: 1) administer pill in person and give take-home doses with instructions (100mg three times a day (TID); subjects will be asked to self-administer the take-home doses at approximately 6-hour intervals); 2) screen for adverse medication effects (Adverse Events Form; heart rate and blood pressure); 3) check for nicotine abstinence compliance (expired CO; urine cotinine; self-reported cigarettes per day); 4) collect self-report measures of withdrawal, smoking urges and mood, which are hypothesized to fluctuate with abstinence and/or medication.

Medication Initiation and Stabilization Phase (Medication Days 1-5): For the first five days of medication, subjects will be told they may smoke their cigarettes as much as they choose (*ad libitum*). They will be asked to attend one brief session per day on Medication Days 1-2. These first days will allow the participants to achieve medication optimal dosing and steady state levels prior to initiating the abstinence period. For Medication Days 3-5, the subjects will maintain the same dose and take the doses at home. During these days, they will have either in person/or phone check-in with a member of the research team to check for reported adverse effects. Brief questions about smoking (e.g., cigarettes per day) and whether they noticed changes in mood or smoking urges will also be asked. These phone check-in days are designed to maintain contact to continue monitoring for safety purposes, while reducing the burden on the participants. Subjects will have an emergency contact so they can access care if needed during this time.

Abstinence Medication Maintenance Sessions (Medication Days 6-7): Beginning on the evening of Medication Day 5, subjects will be asked to refrain from smoking their cigarettes. They will be asked to attend one brief check-in session per day on Medication Days 6-7. Medication doses continue at 100mg TID during this phase. Smoking abstinence will be tracked with CO-levels, urine cotinine screens and self-report.

Post-2 ½ day Abstinence End of Trial Session (Medication Day 8): At the testing session, participants will have been abstinent from smoking for 2 ½ days or more (e.g., 8PM Medication Day 5 through 8AM Medication Day 8), and maintained on tolcapone (or placebo) for the previous week. Participants will take their final dose (100mg) on the morning of Medication Day 8 then complete the same self-report measures and cognitive tasks from the baseline *Ad libitum* Smoking session. Blood will be collected for assessment of nicotine and cotinine (as additional confirmation of abstinence), gonadal hormones and liver function tests (to screen for adverse effects of tolcapone).

Subjects will then complete the Smoking Choice Laboratory Procedure. As we have done in previous studies, participants will complete this paradigm in a designated, negative pressure room. After sample smoking (2 sec cigarette puffs separated by a 20 sec interval) to measure subjective responses to smoking following abstinence, participants exchange tokens for cigarette puffs (2 puffs) or money ($0.75) every 9 minutes until 1.5 hours had elapsed (i.e., 9 min x 10

tokens). This token value is sensitive to increases and decreases in smoking behavior (Sofuoglu *et al*, 2009). If participants choose cigarette puffs instead of money at every opportunity, they will smoke the equivalent of slightly more than one cigarette within the 90-minute laboratory paradigm. Participants will be allowed to decline the sample smoking opportunity if they wish to remain abstinent. Even if they decline the sample smoking opportunity, they will still be asked to remain at the lab for the remainder of the 90-minute paradigm. If they decline this smoking sample opportunity they will also still be allowed to exchange all un-traded tokens for take-home pay (totaling $7.50 if they choose not to trade any tokens for "puffs" of the cigarette) at the end of the 90-minute paradigm.
At the end of this session, participants will stop medication at this time.

1-Week Follow-up Check-in Session: After medication is ended but prior to the in person check-in session, subjects will have the ability to check-in by as needed and will have an emergency contact so they can access care if needed. Approximately one week after the final medication day, participants will be asked to attend a brief in person follow-up check-in session. During this session, they will be asked about reported adverse effects, past week smoking and substance use and whether they noticed changes in mood or smoking urges. They will be asked to complete some questionnaires on these topics. Blood pressure and heart rate will be collected. A urine pregnancy and cotinine screen will be completed. CO levels will be collected. A blood sample will be collected for laboratory tests, including liver functions. Subjects will be discharged at the end of the follow-up session.

Protection of Subjects

In order to participate in a study, each subject must give informed consent. All potential risks will be described in detail to the subjects in the consent form. Confidentiality in this study is of the utmost importance to us. All information obtained will be stored in coded form. The names of the subjects will be used in hospital records.

Confidentiality will be protected by having records identified by code number only with the master list including names kept in a sealed envelope in a locked file in the Principal Investigator's office and by the pharmacy. Subjects will be given telephone numbers to call in case of emergency, 24 hours a day. The potential risk of loss of confidentiality due to data sharing will be minimized by creating de-identified data sets that exclude direct and indirect identifiers. In addition, researchers requesting data will be required to enter into a data sharing agreement. In addition, a Certificate of Confidentiality is being applied for to add some further protections against the research team being compelled to disclose information about participants.

This project will be monitored by the Center's Data and Safety Monitoring Board (DSMB) because the study involves the double-blind treatment of tolcapone and placebo. This board is composed of persons not otherwise affiliated with the clinical study who are experienced in various aspects of the conduct of clinical trials for the treatment of addictive disorders. We propose three investigators located here in Connecticut who are not directly involved in this study – Declan Berry, Ph.D., Sherry McKee, Ph.D., and David Fiellin, M.D. as the members of

the DSMB. The members of the DSMB, and all study Investigators, will complete Conflict of Interest forms created by VA HSS in accordance with NIH guidelines.

Individual Stopping Rules:

For the purposes of participant safety and data quality control, subjects will be discharged from the medication trial if: 1) subject experiences serious side effects which appear related to the study medication including liver toxicity (LFT>2 times greater than the upper limit of the normal range); 2) study physician/medical monitor decides that continued study participation may cause physical or psychological harm to the subject; 3) subject misses 3 consecutive medication-administration study sessions; 4) subject misses 2 consecutive CO level tests, urine cotinine tests during the abstinence phase; 5) subject is found to have used alcohol, illegal drugs or other psychotropic drugs during the medication phase of the study. In the case of any of these causes of early discharge from the trial, participants who began the medication phase of the study- even if they did not complete the medication phase- will still be contacted for phone follow-up and invited to attend the in person follow-up check-in for safety purposes.

Study Stopping Rules:

For the purposes of safety, if more than three subjects are stopped from medication use early due to serious side effects which appear related to the study medication, including but not limited to increased LFTs, twice as high as the upper limit of the normal range, which is not resolved by one-week follow-up, the co-PIs will confer with the DSMB to check whether the entire study should be halted.

Payment:

Subjects will be compensated for participation in each study visit up to a cumulative total of $592.50, with longer visits compensated at a higher rate than brief visits (see details in Initial Review Application). Payments will be made in cash incrementally at each visit as follows: $35 for the initial in-person screen; $100 for the *ad libitum* smoking baseline session; $40 for each of the four in-person check-in visits; for the weekday visit that is labelled an 'in-person' or phone check-in, subjects will be paid $40 if they choose to attend the in-person check-in and they will be paid $10 if they choose the phone check-in for that weekday visit (i.e., either medication day 3 or 5 depending on start date); for weekend phone check-in sessions subjects will always be paid $10 for each of the phone check-ins; $100 for the post-2 ½ day--abstinence session and $50 for the smoking paradigm procedure and $35 for the final one-week follow-up in-person check-in visit. Included in the total possible compensation will be the opportunity to earn an additional $15 for study procedure compliance (e.g., $15 for CO levels <8ppm with no increase in CO across abstinence visits), and the ability to take home money during the Smoking Choice Paradigm wherein smoking opportunities (i.e. 'puffs') are traded for tokens with monetary value. So if no tokens are traded for puffs, the subject can take home up to $7.50 from the paradigm. The compensation schedule is designed to encourage study compliance while avoiding undue coercion to remain in the study. If the subjects choose to terminate a session prematurely, or a session is terminated early for medical reasons, they will receive full payment for that day. If

they become ineligible to continue in the study due to non-compliance with study procedures, they will only be paid for the portions of the study in which they have participated.

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